

10/759,277

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(FILE 'HOME' ENTERED AT 11:03:05 ON 04 OCT 2004)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 11:03:35 ON 04 OCT 2004

L1 185254 S SYNTHETASE?  
L2 1 S "CMP SILAIC ACID"  
L3 11 S "SILAIC ACID"  
L4 0 S L1 AND L3  
L5 69303 S "SIALIC ACID"  
L6 603 S L1 AND L5  
L7 21033 S "CMP"  
L8 438 S L6 AND L7  
L9 6727337 S CLON? OR EXPRESS? OR RECOMBINANT  
L10 182 S L8 AND L9  
E COLEMAN T A/AU  
L11 214 S E3  
E BETENBAUGH M J/AU  
L12 412 S E3-E7  
L13 613 S L11 OR L12  
L14 7 S L10 AND L13  
L15 4 DUP REM L14 (3 DUPLICATES REMOVED)  
L16 29 S HUMAN AND L10  
L17 22 DUP REM L16 (7 DUPLICATES REMOVED)

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NEWS	5	AUG 02	CAplus and CA patent records enhanced with European and Japan Patent Office Classifications
NEWS	6	AUG 02	The Analysis Edition of STN Express with Discover! (Version 7.01 for Windows) now available
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NEWS	12	SEP 14	STN Patent Forum to be held October 13, 2004, in Iselin, NJ
NEWS	13	SEP 27	STANDARDS will no longer be available on STN
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NEWS EXPRESS		JULY 30	CURRENT WINDOWS VERSION IS V7.01, CURRENT MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP), AND CURRENT DISCOVER FILE IS DATED 11 AUGUST 2004
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	ENTRY	SESSION
FULL ESTIMATED COST	0.21	0.21

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FILE 'LIFESCI' ENTERED AT 11:03:35 ON 04 OCT 2004  
 COPYRIGHT (C) 2004 Cambridge Scientific Abstracts (CSA)

=> s synthetase?  
 L1 185254 SYNTHETASE?

=> s "CMP silaia acid"  
 L2 1 "CMP SILAIC ACID"

=> d all

L2 ANSWER 1 OF 1 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN  
 AN 1984:218230 BIOSIS  
 DN PREV198477051214; BA77:51214  
 TI DEFICIENT CMP N ACETYL NEURAMINIC-ACID GLYCO PROTEIN SIALYL TRANSFERASE  
 ACTIVITY IN A CLONE OF KB CELLS WITH ALTERED CELL FUSION ABILITY.  
 AU TOYAMA S [Reprint author]; KOYAMA A H; TOYAMA S  
 CS INST VIRUS RES, KYOTO UNIV, KYOTO 606, JAPAN  
 SO Journal of Biological Chemistry, (1983) Vol. 258, No. 15, pp. 9147-9152.  
 CODEN: JBCHA3. ISSN: 0021-9258.  
 DT Article  
 FS BA  
 LA ENGLISH  
 AB Lines of KB cells resistant to Sendai virus-induced cytolysis were  
 isolated and characterized. The nature of this mutation was studied.  
 Plasma membrane fractions from Sil cells had decreased amount of sialic  
 acid and the same amount of galactose as compared to the membranes from  
 parental KB cells. Sil cells exhibited an increase in sensitivity to  
 toxic effects of ricin and a decrease in sensitivity to wheat germ  
 agglutinin. Binding of wheat germ agglutinin to Sil cells was markedly  
 decreased. Several membrane glycoproteins of Sil cells migrated slightly  
 faster than the corresponding bands of wild type membrane when examined by  
 gel electrophoresis in sodium dodecyl sulfate. Sil cells had decreased  
 sialyltransferase activity that catalyzed the transfer of sialic acid

residues from CMP-N-acetylneuraminic acid to glycoprotein acceptors containing Ga $\beta$ 1  $\rightarrow$  3GalNAc $\alpha$ 1  $\rightarrow$  O-Ser(Thr) [ $\beta$ -galactosidase 1  $\rightarrow$  N-acetylgalactosamine  $\rightarrow$  O-serine (threonine)] chain. The decreased enzyme activity could not be accounted for by the presence of inhibitors, altered pH optimum, or increased sialidase or **CMP-sialic acid** hydrolase activities. A molecular basis for the Sil cell phenotype might be the deficiency of sialyltransferase.

CC Cytology - Human 02508  
 Genetics - Human 03508  
 Biochemistry methods - Proteins, peptides and amino acids 10054  
 Biochemistry methods - Carbohydrates 10058  
 Biochemistry studies - General 10060  
 Biochemistry studies - Nucleic acids, purines and pyrimidines 10062  
 Biochemistry studies - Proteins, peptides and amino acids 10064  
 Biochemistry studies - Carbohydrates 10068  
 Biophysics - Methods and techniques 10504  
 Biophysics - Membrane phenomena 10508  
 Enzymes - Physiological studies 10808  
 Movement 12100  
 Metabolism - Carbohydrates 13004  
 Metabolism - Proteins, peptides and amino acids 13012  
 Metabolism - Nucleic acids, purines and pyrimidines 13014  
 Dental biology - General and methods 19001  
 Toxicology - General and methods 22501  
 Neoplasms - Neoplastic cell lines 24005  
 Virology - Animal host viruses 33506  
 Plant physiology - Chemical constituents 51522  
 Agronomy - Grain crops 52504

IT Major Concepts  
 Cell Biology; Enzymology (Biochemistry and Molecular Biophysics);  
 Genetics; Metabolism; Toxicology; Tumor Biology

IT Miscellaneous Descriptors  
 HUMAN ORAL EPIDERMOID CARCINOMA KB CELL HUMAN ORAL EPIDERMOID CARCINOMA  
 SIL CELL SENDAI VIRUS INDUCED CYTOLYSIS SIALIDASE CMP SIALIC-ACID  
 HYDROLASE RICIN WHEAT GERM AGGLUTININ MEMBRANE GLYCO PROTEIN  
 SIALIC-ACID GALACTOSE GENETIC ENGINEERING

ORGN Classifier  
 Paramyxoviridae 03503  
 Super Taxa  
 Negative Sense ssRNA Viruses; Viruses; Microorganisms  
 Taxa Notes  
 Microorganisms, Negative Sense Single-Stranded RNA Viruses, Viruses

ORGN Classifier  
 Gramineae 25305  
 Super Taxa  
 Monocotyledones; Angiospermae; Spermatophyta; Plantae  
 Taxa Notes  
 Angiosperms, Monocots, Plants, Spermatophytes, Vascular Plants

ORGN Classifier  
 Euphorbiaceae 26055  
 Super Taxa  
 Dicotyledones; Angiospermae; Spermatophyta; Plantae  
 Taxa Notes  
 Angiosperms, Dicots, Plants, Spermatophytes, Vascular Plants

ORGN Classifier  
 Tupaiidae 86245  
 Super Taxa  
 Primates; Mammalia; Vertebrata; Chordata; Animalia  
 Taxa Notes  
 Animals, Chordates, Mammals, Nonhuman Mammals, Nonhuman Vertebrates,  
 Nonhuman Primates, Primates, Vertebrates

RN 3063-71-6 (CMP-N-ACETYLNEURAMINIC-ACID)  
 321976-25-4 (SIALYLTRANSFERASE)

9001-67-6 (SIALIDASE)  
55326-41-5 (CMP-SIALIC-ACID HYDROLASE)  
59-23-4Q (GALACTOSE)  
26566-61-0Q (GALACTOSE)  
9075-81-4 (SIALYL TRANSFERASE)  
50855-33-9Q (GALACTOSE)

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LIFESCI' ENTERED AT 11:03:35 ON 04 OCT 2004

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=> s "silaic acid"

L3 11 "SILAIC ACID"

=> s l1 and l3

L4 0 L1 AND L3

=> s "sialic acid"

L5 69303 "SIALIC ACID"

=> s l1 and l5

L6 603 L1 AND L5

=> s "CMP"

L7 21033 "CMP"

=> s l6 and l7

L8 438 L6 AND L7

=> s clon? or express? or recombinant

5 FILES SEARCHED...

L9 6727337 CLON? OR EXPRESS? OR RECOMBINANT

=> s l8 and l9

L10 182 L8 AND L9

=> e coleman t a/au

E1 1 COLEMAN SYLVIA ETHEL/AU  
E2 382 COLEMAN T/AU  
E3 214 --> COLEMAN T A/AU  
E4 5 COLEMAN T B/AU  
E5 7 COLEMAN T C/AU  
E6 15 COLEMAN T D/AU  
E7 16 COLEMAN T E/AU  
E8 52 COLEMAN T F/AU  
E9 414 COLEMAN T G/AU  
E10 136 COLEMAN T H/AU  
E11 1 COLEMAN T H JR/AU  
E12 105 COLEMAN T J/AU

=> s e3

L11 214 "COLEMAN T A"/AU

=> e betenbaugh m j/au

E1 1 BETENBAUGH H S/AU  
E2 16 BETENBAUGH M/AU  
E3 245 --> BETENBAUGH M J/AU  
E4 2 BETENBAUGH M J \*/AU

E5	14	BETENBAUGH MICHAEL/AU
E6	150	BETENBAUGH MICHAEL J/AU
E7	1	BETENBAUGH MICHAEL JOSEPH/AU
E8	3	BETENBAUGH MJ/AU
E9	3	BETENBAUGH T M/AU
E10	5	BETENCOURT A/AU
E11	3	BETENCOURT ALAIN/AU
E12	1	BETENCOURT J C A/AU

=> s e3-e7

L12 412 ("BETENBAUGH M J"/AU OR "BETENBAUGH M J \*"/AU OR "BETENBAUGH MICHAEL"/AU OR "BETENBAUGH MICHAEL J"/AU OR "BETENBAUGH MICHAEL JOSEPH"/AU)

=> s l11 or l12

L13 613 L11 OR L12

=> d his

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L3	11	S "SILAIC ACID"
L4	0	S L1 AND L3
L5	69303	S "SIALIC ACID"
L6	603	S L1 AND L5
L7	21033	S "CMP"
L8	438	S L6 AND L7
L9	6727337	S CLON? OR EXPRESS? OR RECOMBINANT
L10	182	S L8 AND L9
		E COLEMAN T A/AU
L11	214	S E3
		E BETENBAUGH M J/AU
L12	412	S E3-E7
L13	613	S L11 OR L12

=> s l10 and l13

L14 7 L10 AND L13

=> dup rem l14

PROCESSING COMPLETED FOR L14

L15 4 DUP REM L14 (3 DUPLICATES REMOVED)

=> d 1-4 ibib ab

L15 ANSWER 1 OF 4 BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN

ACCESSION NUMBER: 2003-06688 BIOTECHDS

TITLE: Manipulating glycoprotein production in insect cell, involves enhancing **expression** of enzymes involved in carbohydrate processing pathway such as N-acetylglucosamine-2 epimerase or **sialic acid synthetase**;

**recombinant** protein production via plasmid **expression** in host cell for use in diagnosis and therapy

AUTHOR: **BETENBAUGH M J**; LAWRENCE S; LEE Y C; **COLEMAN T A**

PATENT ASSIGNEE: **BETENBAUGH M J**; LAWRENCE S; LEE Y C; **COLEMAN T A**

PATENT INFO: US 2002142386 3 Oct 2002

APPLICATION INFO: US 2001-930440 16 Aug 2001

PRIORITY INFO: US 2001-930440 16 Aug 2001; US 1999-122582 2 Mar 1999

DOCUMENT TYPE: Patent  
LANGUAGE: English  
OTHER SOURCE: WPI: 2003-102519 [09]  
AB DERWENT ABSTRACT:

NOVELTY - Manipulating (M1) glycoprotein production in an insect cell comprising enhancing **expression** of an enzyme (E) such as N-acetylglucosamine-2 (GlcNAc-2) epimerase, one catalyzing conversion of UDP-GlcNAc to mannose (Man)NAc, **sialic acid synthetase**, aldolase, cytidine monophosphate-**sialic acid (CMP-SA) synthetase** or **CMP-SA transporter**, where the **expression** of each (E) is enhanced to above endogenous levels, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) a cell of interest (I) producing the donor substrate **CMP-SA** above endogenous levels; (2) a cell of interest (II) producing an acceptor substrate, the donor substrate **CMP-SA**, and **expressing** the enzyme sialyltransferase, where the acceptor substrate is a glycan; (3) a cell of interest (III) producing sialylated glycoprotein above endogenous levels; (4) a kit (IV) for **expression** of sialylated glycoproteins, comprising (I); (5) producing (M2) sialylated glycoproteins, by **expressing** a heterologous protein in an insect cell manipulated by M1; and (6) producing (M3) sialylated glycoprotein in a cell of interest, by determining the carbohydrate substrates in the cell, transforming the cell with enzymes to produce necessary precursor substrates, and constructing a processing pathway in the cell to produce a sialylated glycoprotein.

WIDER DISCLOSURE - Disclosed are: (A) an assay for sialylation; (B) sequence variants of the amino acid sequence or nucleotide sequence of human aldolase, human **CMP-SA synthetase** or **SA-synthetase**, and their fragments; (C) nucleic acid molecules encoding the above mentioned variant polypeptides; (D) polynucleotides having a lower degree of identity but having sufficient similarity to (E) so as to perform one or more of the same functions as (E); (E) **recombinant** vectors including isolated nucleic acid molecules encoding (E); (F) a host cell comprising the above mentioned vector and/or nucleic acid molecule; and (G) **expressing** heterologous proteins in (I), (II) or (III).

BIOTECHNOLOGY - Preferred Method: In M1, the GlcNAc-2 epimerase, the enzyme catalyzing conversion of UDP-GlcNAc to ManNAc, or **CMP-SA synthetase** is a human enzyme. The **expression** of (E) is enhanced by M1. The **sialic acid synthetase** has a sequence of 359 amino acids fully defined in the specification, and the aldolase has a sequence of 230 or 434 amino acids fully defined in the specification. M1 further comprises enhancing the **expression** of at least one enzyme selected from Gal T, GlcNAc TI, GlcNAc TII and sialyltransferase. M1 further comprises suppressing the activity of endogenous N-acetylglucosaminidase. In M2, the heterologous protein is a mammalian protein selected from plasminogen, transferrin, Na<sup>+</sup>, K<sup>+</sup>-ATPase or thyrotropin. In M3, the cell is a yeast, insect, fungal, plant or bacterial cell. The cell enhances the **expression** of both **sialic acid synthetase** and **CMP-SA synthetase**. Preferred Cell: In (II), the glycan is a branched glycan comprising GalGlcNAcMan by at least one branch of the glycan and the Gal is a terminal Gal. The glycan is an asparagine-linked glycan. In (III), the glycoprotein is asparagine (N)-linked, and the glycoprotein is a mammalian heterologous selected from plasminogen, transferrin, Na<sup>+</sup>, K<sup>+</sup>-ATPase, and thyrotropin. (I) **expresses** (E).

ACTIVITY - None given.

MECHANISM OF ACTION - Vaccine. No suitable data given.

USE - M1 is useful for manipulating glycoprotein production in an insect cell. M2 or M3 is useful for producing sialylated glycoprotein (claimed). The sialylated glycoprotein produced by the above mentioned methods are useful as pharmaceutical compositions, vaccines, diagnostics

and therapeutics.

EXAMPLE - No suitable example given. (88 pages)

L15 ANSWER 2 OF 4 BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN  
DUPLICATE 1

ACCESSION NUMBER: 2001-14234 BIOTECHDS

TITLE: Cells producing cytidine monophosphate-**sialic acid** and sialylated glycoprotein above endogenous levels for production of vaccines and therapeutics; metabolic engineering for **recombinant** vaccine production

AUTHOR: **Betenbaugh M J**; Lawrence S; Lee Y C; **Coleman T A**; Palter K; Jarvis D

PATENT ASSIGNEE: Hum.Genome-Sci.; Univ.Johns-Hopkins; Univ.Temple; Univ.Wyoming

LOCATION: Rockville, MD, USA; Baltimore, MD, USA; Philadelphia, PA, USA; Laramie, WY, USA.

PATENT INFO: WO 2001042492 14 Jun 2001

APPLICATION INFO: WO 2000-US33136 7 Dec 2000

PRIORITY INFO: US 1999-169839 9 Dec 1999

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2001-441575 [47]

AB Cells (C1 and C2) where C1 produces the donor substrate cytidine monophosphate-**sialic acid** (**CMP-SA**) above endogenous levels and C2 produces sialylated glycoprotein above endogenous levels, are claimed. Also claimed are: a kit for **expressing** sialylated glycoproteins comprising C1 or C2; manipulating glycoprotein production in an insect cell involving enhancing N-acetylglucosamine-2-epimerase, an enzyme catalyzing conversion of UDP-N-acetylglucosamine to N-**CMP-SA-synthetase** or **CMP-SA-transporter** above endogenous levels; producing sialylated glycoproteins comprising **expressing** a heterologous protein in an insect cell manipulated according to the method; producing sialylated glycoprotein in a cell by determining the carbohydrate substrates in a cell, transforming the cell with enzymes to give necessary precursor substrates, and constructing a processing pathway in the cell to produce a sialylated glycoprotein. The glycoproteins are useful in vaccines and as diagnostic tools. (182pp)

L15 ANSWER 3 OF 4 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED.  
on STN DUPLICATE 2

ACCESSION NUMBER: 2001412468 EMBASE

TITLE: **Cloning** and **expression** of human **sialic acid** pathway genes to generate **CMP-sialic acids** in insect cells.

AUTHOR: Lawrence S.M.; Huddleston K.A.; Tomiya N.; Nguyen N.; Lee Y.C.; Vann W.F.; **Coleman T.A.**; **Betenbaugh M.J.**

CORPORATE SOURCE: M.J. Betenbaugh, Department of Chemical Engineering, Johns Hopkins University, 3400 N. Charles St., Baltimore, MD 21218, United States. beten@jhu.edu

SOURCE: Glycoconjugate Journal, (2001) 18/3 (205-213).  
Refs: 38  
ISSN: 0282-0080 CODEN: GLJOEW

COUNTRY: Netherlands

DOCUMENT TYPE: Journal; Article

FILE SEGMENT: 004 Microbiology

LANGUAGE: English

SUMMARY LANGUAGE: English

AB The addition of **sialic acid** residues to glycoproteins can affect important protein properties including biological activity and in vivo circulatory half-life. For sialylation to occur, the donor sugar

nucleotide cytidine monophospho-**sialic acid** (**CMP-SA**) must be generated and enzymatically transferred to an acceptor oligosaccharide. However, examination of insect cells grown in serum-free medium revealed negligible native levels of the most common **sialic acid** nucleotide, **CMP-N-acetylneuraminic acid (CMP-Neu5Ac)**. To increase substrate levels, the enzymes of the metabolic pathway for **CMP-SA** synthesis have been engineered into insect cells using the baculovirus **expression** system. In this study, a human **CMP-sialic acid** synthase cDNA was identified and found to encode a protein with 94% identity to the murine homologue. The human **CMP-sialic acid** synthase (**Cmp-Sas**) is ubiquitously **expressed** in human cells from multiple tissues. When **expressed** in insect cells using the baculovirus vector, the encoded protein is functional and localizes to the nucleus as in mammalian cells. In addition, co-**expression** of **Cmp-Sas** with the recently **cloned** **sialic acid** phosphate synthase with N-acetylmannosamine feeding yields intracellular **CMP-Neu5Ac** levels 30 times higher than those observed in unsupplemented CHO cells. The absence of any one of these three components abolishes **CMP-Neu5Ac** production in vivo. However, when N-acetylmannosamine feeding is omitted, the sugar nucleotide form of deaminated Neu5Ac, **CMP-2-keto-3-deoxy-D-glycero-D-galacto-nononic acid (CMP-KDN)**, is produced instead, indicating that alternative **sialic acid** glycoforms may eventually be possible in insect cells. The human **CMP-SAS** enzyme is also capable of **CMP-N-glycolylneuraminic acid (CMP-Neu5Gc)** synthesis when provided with the proper substrate. Engineering the **CMP-SA** metabolic pathway may be beneficial in various cell lines in which **CMP-Neu5Ac** production limits sialylation of glycoproteins or other glycans.

L15 ANSWER 4 OF 4 BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN  
DUPLICATE 3

ACCESSION NUMBER: 2000-14572 BIOTECHDS

TITLE: **Recombinant** production of sialylated glycoproteins using cells in which the **expression** of enzyme, e.g. **sialic acid-synthetase**, involved in the sialylation reaction has been altered; production of sialylated glycoprotein

AUTHOR: **Betenbaugh M J**; Lawrence S; Lee Y C; Jarvis D; **Coleman T A**

PATENT ASSIGNEE: Hum.Genome-Sci.; Univ.Johns-Hopkins; Univ.Wyoming

LOCATION: Rockville, MD, USA; Baltimore, MD, USA; Laramie, WY, USA.

PATENT INFO: WO 2000052135 8 Sep 2000

APPLICATION INFO: WO 2000-US5313 1 Mar 2000

PRIORITY INFO: US 990169624 8 Dec 1999; US 1999-122582 2 Mar 1999

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2000-572178 [53]

AB Methods and recombinantly engineered cells for producing glycoproteins having sialylated oligosaccharides is claimed. The methods involve altering the **expression** of enzymes involved in carbohydrate processing e.g. **sialic-acid-synthetase**. Also claimed are: a cell (I) producing the donor substrate cytidine monophosphate-**sialic acid (CMP-SA)** above endogenous levels; a kit (II) for **expression** of sialylated glycoprotein, containing (I); a method (III) for manipulating glycoprotein production in an insect cell by enhancing **expression** of at least one enzyme; a method (IV) for producing sialylated glycoprotein by **expressing** a heterologous protein in an insect cell manipulated via (III); and a method (V) for producing a sialylated glycoprotein in a cell of interest by determining the carbohydrate substances in the cell, transforming the cell with enzyme to produce necessary precursor substrates, and constructing a processing pathway in

the cell to produce a sialylated glycoprotein. The methods and cells may be used for producing sialylate glycoproteins. (144pp)

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L15 4 DUP REM L14 (3 DUPLICATES REMOVED)

=> s human and l10

L16 29 HUMAN AND L10

=> dup rem l16

PROCESSING COMPLETED FOR L16

L17 22 DUP REM L16 (7 DUPLICATES REMOVED)

=> d 1-22 ibib ab

L17 ANSWER 1 OF 22 BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN

ACCESSION NUMBER: 2004-18268 BIOTECHDS

TITLE: Producing glycoprotein with animal type sugar chain, comprises introducing gene encoding enzyme that adds **sialic acid** to non-reducing terminal of sugar chain, and gene of heterologous protein, into plant cell, cultivating plant cell;  
transgenic plant construction via bacterium-mediated transformation for use in protein production

AUTHOR: FUJIYAMA K; SEKI T

PATENT ASSIGNEE: FUJIYAMA K; SEKI T

PATENT INFO: WO 2004063370 29 Jul 2004

APPLICATION INFO: WO 2004-P 264 15 Jan 2004

PRIORITY INFO: JP 2003-7687 15 Jan 2003; JP 2003-7687 15 Jan 2003

DOCUMENT TYPE: Patent

LANGUAGE: Japanese

OTHER SOURCE: WPI: 2004-561900 [54]

AB DERWENT ABSTRACT:

NOVELTY - Producing (M1) glycoprotein (I) having animal type sugar chain, involves introducing a gene encoding the enzyme that can add **sialic acid** to the non-reducing terminal of sugar chain, and the gene of heterologous protein, into the plant cell, cultivating the transformed plant cell, and recovering the culture solution of the plant cell.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) plant cell (II) transformed with the gene that codes **sialic acid synthetase, CMP-**

**sialic acid synthetase** and/or **CMP-sialic acid** transporter exhibiting saccharide addition mechanism (adding **sialic acid** to the non-reducing terminal of the sugar chain of glycoprotein), where (II) can take in the precursor of **sialic acid** or **sialic acid**, and has a vesicle that allows the uptake of **sialic acid**; and (2) plant body regenerate from (II).

BIOTECHNOLOGY - Preferred Method: In (M1), the glycoprotein having animal type sugar chain contains a core sugar chain and the external sugar chain, where the core sugar chain essentially comprises several numbers of mannose and acetylglucosamine, while the external sugar chain has a terminal sugar chain moiety containing a non-reducing end galactose. The external sugar chain is the linear or mono, tri or tetra branched sugar chain.

USE - (M1) is useful for producing glycoprotein having animal type sugar chain (claimed).

EXAMPLE - The DNA of the Escherichia coli was used as the template, PCR was performed using the primers having the sequences such as 5'-tttagctcgagacaatgagtaatatatat-3' and 5'-ttttctcgagttattattccccctgatttttaaattc-3', the obtained PCR product was digested by XhoI and SalI restriction enzymes, and the neuB fragment was obtained. The DNA of the Nicotiana tabacum cv SRI was used as the template, PCR was performed using the primers having the sequences such as 5'-tttaagtcgacacgatgagagg-3' and 5'-aatcgtcgacccttaactgtc-3', the obtained PCR product was digested by restriction enzymes. The obtained neuB fragment, and the **CMP-sialic acid** transporter (CST) gene were connected, and the target CTS-neuB gene was obtained. The obtained CTS-neuB gene was introduced into the plasmid pBI221, and the vector pBI121-CTS-neuB was obtained. The XhoI and SpeI restriction enzyme site was introduced into the vector pBI121-CTS-neuB by PCR amplification using specific primers, and the plasmid containing the **expression** cassette of CTS-neuB gene was obtained. The cDNA of the **human** kidney was used as the template, PCR was performed using the primers having the sequences such as 5'-gttactagtagtgactcgggtggagaaggggcccacctccgtcctcaacccgcggggcgaccgtccc-3' and 5'-tgaggagctcctatttttggcatgaattatt-3', the obtained PCR product was introduced into the plasmid pBI221. The obtained **expression** cassette was then introduced into pGPTV-HPT, and the pGPTV-HPT-hCSS was obtained. The XhoI and SpeI restriction enzyme site was introduced into the vector pGPTV-HPT-hCSS by PCR amplification using specific primers, and the plasmid containing the **expression** cassette of HPT-hCSS gene was obtained. The plasmid containing the **expression** cassette of HPT-hCST was also obtained. The plasmids containing the **expression** cassettes of CST-neuB gene and the HPT-hCST gene were digested by XhoI and SpeI enzymes, and both the XhoI/SpeI fragments were introduced into pGEM-T Easy vector, and the plasmid pGEM-T-hCST-CTS-neuB was obtained. To the plasmid pGEM-T-hCST-CTS-neuB gene, the **expression** cassette of HPT-hCSS was introduced, and the plasmid pGPTV-HPT-hCSS-hCST-CTS-neuB was obtained. The pGPTV-HPT-hCSS-hCST-CTS-neuB was introduced into Agrobacterium tumefaciens LBA4404. The transformed LBA4404 was allowed to infect the cell of tobacco. The infected tobacco cell was cultivated for 7 days, the DNA was isolated from the plant cell, and the presence of the **CMP-sialic acid synthetase** (CSS) and **CMP-sialic acid** transporter (CST) gene was detected. The results showed the presence of CSS and CST genes in the transformed plant cell. Thus the plant cell containing CST and CSS in its genome was obtained. (88 pages)

L17 ANSWER 2 OF 22 MEDLINE on STN  
ACCESSION NUMBER: 2004336041 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 15238249  
TITLE: **CMP-sialic acid synthetase** of the nucleus.  
AUTHOR: Kean Edward L; Munster-Kuhnel Anja K; Gerardy-Schahn Rita

CORPORATE SOURCE: Department of Ophthalmology, Case Western Reserve University, Cleveland, OH 4410, USA.  
 SOURCE: Biochimica et biophysica acta, (2004 Jul 6) 1673 (1-2) 56-65. Ref: 73  
 Journal code: 0217513. ISSN: 0006-3002.  
 PUB. COUNTRY: Netherlands  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 General Review; (REVIEW)  
 (REVIEW, TUTORIAL)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 200408  
 ENTRY DATE: Entered STN: 20040708  
 Last Updated on STN: 20040818  
 Entered Medline: 20040817

AB **Sialic acids** of cell surface glycoconjugates play a pivotal role in the structure and function of animal cells and in some bacterial pathogens. The pattern of cell surface sialylation is species specific, and, in the animal, highly regulated during embryonic development. A prerequisite for the synthesis of sialylated glycoconjugates is the availability of the activated sugar-nucleotide cytidine 5'-monophosphate N-acetylneuraminic acid (**CMP-NeuAc**), which provides the substrate for sialyltransferases. Trials to purify the enzymatic activity responsible for the synthesis of **CMP-NeuAc** from different animal sources demonstrated that the major localisation of the enzyme is the cell nucleus. These earlier findings were confirmed when the murine **CMP-NeuAc synthetase** was **cloned** and the subcellular transport of **recombinant** epitope tagged forms visualised by indirect immunofluorescence. Today, the primary sequence elements that direct murine **CMP-NeuAc synthetase** into the cell nucleus are known, however, information regarding the physiological relevance of the nuclear destination is still not available. With this article, we provide a detailed review on earlier and recent findings that identified and confirmed the unusual subcellular localisation of the **CMP-NeuAc synthetase**. In addition, we take the advantage to discuss most recent developments towards understanding structure--function relations of this enzyme.

L17 ANSWER 3 OF 22 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2003:698614 HCAPLUS  
 DOCUMENT NUMBER: 139:228058  
 TITLE: **Human** genes and proteins involved regulation of angiogenesis and their use in drug screening, diagnosis, and therapy  
 INVENTOR(S): Colin, Sylvie; Schneider, Christophe; Al Mahmood, Salman  
 PATENT ASSIGNEE(S): Gene Signal, Fr.  
 SOURCE: Fr. Demande, 405 pp.  
 CODEN: FRXXBL  
 DOCUMENT TYPE: Patent  
 LANGUAGE: French  
 FAMILY ACC. NUM. COUNT: 2  
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
FR 2836686	A1	20030905	FR 2002-2717	20020304
FR 2836687	A1	20030905	FR 2002-4546	20020411
WO 2003074073	A2	20030912	WO 2003-FR695	20030304

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ,

UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD,  
RU, TJ, TM  
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG,  
CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC,  
NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ,  
GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.:

FR 2002-2717 A 20020304

FR 2002-4546 A 20020411

AB **Human** genes and the corresponding proteins which are implicated in regulation of angiogenesis, antisense oligonucleotides complementary to these nucleic acids, antibodies to the proteins, and transgenic cells under- or overexpressing these genes are disclosed. The angiogenesis-related nucleic acids and proteins, antibodies, and transgenic cells **expressing** the angiogenesis-related nucleic acid may be used in diagnosis and therapy and in screening for angiogenesis-regulating compds. Vectors containing the angiogenesis-related nucleic acid and transgenic cells producing the encoded proteins are further disclosed. Thus, using a subtractive hybridization procedure, 54 genes the **expression** of which is altered during angiogenesis were identified.

L17 ANSWER 4 OF 22 BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN

ACCESSION NUMBER: 2003-06688 BIOTECHDS

TITLE: Manipulating glycoprotein production in insect cell, involves enhancing **expression** of enzymes involved in carbohydrate processing pathway such as N-acetylglucosamine-2 epimerase or **sialic acid synthetase**;  
**recombinant** protein production via plasmid  
**expression** in host cell for use in diagnosis and therapy

AUTHOR: BETENBAUGH M J; LAWRENCE S; LEE Y C; COLEMAN T A

PATENT ASSIGNEE: BETENBAUGH M J; LAWRENCE S; LEE Y C; COLEMAN T A

PATENT INFO: US 2002142386 3 Oct 2002

APPLICATION INFO: US 2001-930440 16 Aug 2001

PRIORITY INFO: US 2001-930440 16 Aug 2001; US 1999-122582 2 Mar 1999

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2003-102519 [09]

AB DERWENT ABSTRACT:

NOVELTY - Manipulating (M1) glycoprotein production in an insect cell comprising enhancing **expression** of an enzyme (E) such as N-acetylglucosamine-2 (GlcNAc-2) epimerase, one catalyzing conversion of UDP-GlcNAc to mannose (Man)NAC, **sialic acid synthetase**, aldolase, cytidine monophosphate-**sialic acid** (CMP-SA) **synthetase** or **CMP-SA** transporter, where the **expression** of each (E) is enhanced to above endogenous levels, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) a cell of interest (I) producing the donor substrate **CMP-SA** above endogenous levels; (2) a cell of interest (II) producing an acceptor substrate, the donor substrate **CMP-SA**, and **expressing** the enzyme sialyltransferase, where the acceptor substrate is a glycan; (3) a cell of interest (III) producing sialylated glycoprotein above endogenous levels; (4) a kit (IV) for **expression** of sialylated glycoproteins, comprising (I); (5) producing (M2) sialylated glycoproteins, by **expressing** a heterologous protein in an insect cell manipulated by M1; and (6) producing (M3) sialylated glycoprotein in a cell of interest, by determining the carbohydrate substrates in the cell, transforming the cell with enzymes to produce necessary precursor substrates, and constructing a processing pathway in the cell to produce a sialylated glycoprotein.

WIDER DISCLOSURE - Disclosed are: (A) an assay for sialylation; (B)

sequence variants of the amino acid sequence or nucleotide sequence of human aldolase, human **CMP-SA synthetase** or **SA-synthetase**, and their fragments; (C) nucleic acid molecules encoding the above mentioned variant polypeptides; (D) polynucleotides having a lower degree of identity but having sufficient similarity to (E) so as to perform one or more of the same functions as (E); (E) **recombinant** vectors including isolated nucleic acid molecules encoding (E); (F) a host cell comprising the above mentioned vector and/or nucleic acid molecule; and (G) **expressing** heterologous proteins in (I), (II) or (III).

BIOTECHNOLOGY - Preferred Method: In M1, the GlcNAc-2 epimerase, the enzyme catalyzing conversion of UDP-GlcNAc to ManNAc, or **CMP-SA synthetase** is a human enzyme. The **expression** of (E) is enhanced by M1. The **sialic acid synthetase** has a sequence of 359 amino acids fully defined in the specification, and the aldolase has a sequence of 230 or 434 amino acids fully defined in the specification. M1 further comprises enhancing the **expression** of at least one enzyme selected from Gal T, GlcNAc TI, GlcNAc TII and sialyltransferase. M1 further comprises suppressing the activity of endogenous N-acetylglucosaminidase. In M2, the heterologous protein is a mammalian protein selected from plasminogen, transferrin, Na<sup>+</sup>, K<sup>+</sup>-ATPase or thyrotropin. In M3, the cell is a yeast, insect, fungal, plant or bacterial cell. The cell enhances the **expression** of both **sialic acid synthetase** and **CMP-SA synthetase**. Preferred Cell: In (II), the glycan is a branched glycan comprising GalGlcNAcMan by at least one branch of the glycan and the Gal is a terminal Gal. The glycan is an asparagine-linked glycan. In (III), the glycoprotein is asparagine (N)-linked, and the glycoprotein is a mammalian heterologous selected from plasminogen, transferrin, Na<sup>+</sup>, K<sup>+</sup>-ATPase, and thyrotropin. (I) **expresses** (E).

ACTIVITY - None given.

MECHANISM OF ACTION - Vaccine. No suitable data given.

USE - M1 is useful for manipulating glycoprotein production in an insect cell. M2 or M3 is useful for producing sialylated glycoprotein (claimed). The sialylated glycoprotein produced by the above mentioned methods are useful as pharmaceutical compositions, vaccines, diagnostics and therapeutics.

EXAMPLE - No suitable example given. (88 pages)

L17 ANSWER 5 OF 22 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2001:435294 HCAPLUS

DOCUMENT NUMBER: 135:41800

TITLE: **Recombinant** cells with altered intracellular sialylation pathways and their use in producing glycoproteins

INVENTOR(S): Betenbaugh, Michael J.; Lawrence, Shawn; Lee, Yuan C.; Coleman, Timothy A.; Palter, Karen; Jarvis, Don

PATENT ASSIGNEE(S): Human Genome Sciences, Inc, USA; Johns Hopkins University; Temple University; University of Wyoming

SOURCE: PCT Int. Appl., 182 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001042492	A1	20010614	WO 2000-US33136	20001207
W:	AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU,			

SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN,  
YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM  
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY,  
DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF,  
BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 1999-169839P P 19991209

AB Methods for manipulating carbohydrate processing pathways in cells of interest are provided. Methods are directed at manipulating multiple pathways involved with the sialylation reaction by using **recombinant** DNA technol. and substrate feeding approaches to enable the production of sialylated glycoproteins in cells of interest. These carbohydrate engineering efforts encompass the implementation of new carbohydrate bioassays, the examination of a selection of insect cell lines and the use of bioinformatics to identify gene sequences for critical processing enzymes. The compns. comprise cells of interest producing sialylated glycoproteins. The methods and compns. are useful for heterologous **expression** of glycoproteins. Thus, the cDNA for a **human sialic acid 9-phosphate synthetase** which produces phosphorylated KDN and Neu5Ac from ManNAc-6-P and Man-6-P was **cloned** and sequenced. Sf9 cells infected with a baculovirus encoding this enzymes produced enhanced levels of **sialic acids** when the culture medium was supplemented with ManNAc.

REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L17 ANSWER 6 OF 22 MEDLINE on STN DUPLICATE 1

ACCESSION NUMBER: 2001467579 MEDLINE

DOCUMENT NUMBER: PubMed ID: 11479279

TITLE: Molecular **cloning** of a unique **CMP-sialic acid synthetase** that effectively utilizes both deaminoneuraminic acid (KDN) and N-acetylneuraminic acid (Neu5Ac) as substrates.  
AUTHOR: Nakata D; Munster A K; Gerardy-Schahn R; Aoki N; Matsuda T; Kitajima K

CORPORATE SOURCE: Department of Applied Molecular Biosciences, Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya 464-8601, Japan.

SOURCE: Glycobiology, (2001 Aug) 11 (8) 685-92.  
Journal code: 9104124. ISSN: 0959-6658.

PUB. COUNTRY: England: United Kingdom

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

OTHER SOURCE: GENBANK-AB027414

ENTRY MONTH: 200110

ENTRY DATE: Entered STN: 20010830  
Last Updated on STN: 20011015  
Entered Medline: 20011011

AB 2-keto-3-deoxy-D-glycero-D-galacto-nononic acid (KDN) is a **sialic acid** (Sia) that is ubiquitously **expressed** in vertebrates during normal development and tumorigenesis. Its **expression** is thought to be regulated by multiple biosynthetic steps catalyzed by several enzymes, including **CMP-Sia synthetase**. Using crude enzyme preparations, it was shown that mammalian **CMP-Sia synthetases** had very low activity to synthesize **CMP-KDN** from KDN and CTP, and the corresponding enzyme from rainbow trout testis had high activity to synthesize both **CMP-KDN** and **CMP-N-acetylneuraminic acid (Neu5Ac)** (Terada et al. [1993] J. Biol. Chemical, 268, 2640-2648). To demonstrate if the unique substrate specificity found in the crude trout enzyme is conveyed by a single enzyme, cDNA **cloning** of trout **CMP-Sia synthetase** was carried out by PCR-based strategy. The trout enzyme was shown to consist of 432 amino acids with two potential nuclear localization signals, and the cDNA sequence displayed 53.8% identity to that of the murine enzyme.

Based on the Vmax/Km values, the **recombinant** trout enzyme had high activity toward both KDN and Neu5Ac (1.1 versus 0.68 min<sup>-1</sup>). In contrast, the **recombinant** murine enzyme had 15 times lower activity toward KDN than Neu5Ac (0.23 versus 3.5 min<sup>-1</sup>). Northern blot analysis suggested that several sizes of the mRNA are **expressed** in testis, ovary, and liver in a tissue-specific manner. These results indicate that at least one **cloned** enzyme has the ability to utilize both KDN and Neu5Ac as substrates efficiently and is useful for the production of **CMP-KDN**.

L17 ANSWER 7 OF 22 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED.  
on STN DUPLICATE 2

ACCESSION NUMBER: 2001412468 EMBASE  
TITLE: **Cloning and expression of human sialic acid pathway genes to generate CMP-sialic acids in insect cells.**  
AUTHOR: Lawrence S.M.; Huddleston K.A.; Tomiya N.; Nguyen N.; Lee Y.C.; Vann W.F.; Coleman T.A.; Betenbaugh M.J.  
CORPORATE SOURCE: M.J. Betenbaugh, Department of Chemical Engineering, Johns Hopkins University, 3400 N. Charles St., Baltimore, MD 21218, United States. beten@jhu.edu  
SOURCE: Glycoconjugate Journal, (2001) 18/3 (205-213).  
Refs: 38  
ISSN: 0282-0080 CODEN: GLJOEW  
COUNTRY: Netherlands  
DOCUMENT TYPE: Journal; Article  
FILE SEGMENT: 004 Microbiology  
LANGUAGE: English  
SUMMARY LANGUAGE: English

AB The addition of **sialic acid** residues to glycoproteins can affect important protein properties including biological activity and in vivo circulatory half-life. For sialylation to occur, the donor sugar nucleotide cytidine monophospho-**sialic acid** (**CMP-SA**) must be generated and enzymatically transferred to an acceptor oligosaccharide. However, examination of insect cells grown in serum-free medium revealed negligible native levels of the most common **sialic acid** nucleotide, **CMP-N-acetylneuraminic acid** (**CMP-Neu5Ac**). To increase substrate levels, the enzymes of the metabolic pathway for **CMP-SA** synthesis have been engineered into insect cells using the baculovirus **expression** system. In this study, a **human CMP-sialic acid** synthase cDNA was identified and found to encode a protein with 94% identity to the murine homologue. The **human CMP-sialic acid** synthase (**Cmp-Sas**) is ubiquitously **expressed** in **human** cells from multiple tissues. When **expressed** in insect cells using the baculovirus vector, the encoded protein is functional and localizes to the nucleus as in mammalian cells. In addition, co-**expression** of **Cmp-Sas** with the recently **cloned sialic acid** phosphate synthase with N-acetylmannosamine feeding yields intracellular **CMP-Neu5Ac** levels 30 times higher than those observed in unsupplemented CHO cells. The absence of any one of these three components abolishes **CMP-Neu5Ac** production in vivo. However, when N-acetylmannosamine feeding is omitted, the sugar nucleotide form of deaminated Neu5Ac, **CMP-2-keto-3-deoxy-D-glycero-D-galacto-nononic acid** (**CMP-KDN**), is produced instead, indicating that alternative **sialic acid** glycoforms may eventually be possible in insect cells. The **human CMP-SAS** enzyme is also capable of **CMP-N-glycolylneuraminic acid** (**CMP-Neu5Gc**) synthesis when provided with the proper substrate. Engineering the **CMP-SA** metabolic pathway may be beneficial in various cell lines in which **CMP-Neu5Ac** production limits sialylation of glycoproteins or other glycans.

L17 ANSWER 8 OF 22 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on  
STN

ACCESSION NUMBER: 2002:189008 BIOSIS  
DOCUMENT NUMBER: PREV200200189008  
TITLE: Sialylation of the Pasteurella multocida cell surface.  
AUTHOR(S): Vimr, E. R. [Reprint author]; Lichtensteiger, C. A.  
[Reprint author]  
CORPORATE SOURCE: University of Illinois at Urbana-Champaign, Urbana, IL, USA  
SOURCE: Abstracts of the General Meeting of the American Society  
for Microbiology, (2001) Vol. 101, pp. 141. print.  
Meeting Info.: 101st General Meeting of the American  
Society for Microbiology. Orlando, FL, USA. May 20-24,  
2001. American Society for Microbiology.  
ISSN: 1060-2011.  
DOCUMENT TYPE: Conference; (Meeting)  
Conference; Abstract; (Meeting Abstract)  
Conference; (Meeting Poster)  
LANGUAGE: English  
ENTRY DATE: Entered STN: 13 Mar 2002  
Last Updated on STN: 13 Mar 2002

AB Bacterial pathogens belonging to the Haemophilus-Actinobacillus-  
Pasteurella (HAP) group are obligate microparasites of the mammalian  
oropharynx and can cause severe respiratory or invasive disease in  
**humans**, domestic animals, and wildlife. **Sialic**  
**acids** are ubiquitous components of mammalian cell surfaces and  
serum glycoconjugates. At least one HAP member, Haemophilus influenzae,  
has been shown to mimic the host environment by phase-variation of its  
surface **sialic acids**. To our knowledge, no other HAP  
member has been shown to sialylate its cell surface. However, recent DNA  
sequencing of the Pasteurella multocida genome suggests this bacterium may  
encode functions for **sialic acid** catabolism,  
activation (synthesis of **CMP-sialic acid**),  
and glycosyl transfer ( $\alpha$ 2,6-sialyltransferase). To determine if P.  
multocida is capable of sialylation, the cell-free membrane fractions from  
two common serotypes (types A and D) were shown to sialylate endogenous  
acceptor(s) when provided with exogenous **CMP-(14C)sialic**  
**acid**. Confirmation that the transferred **sialic**  
**acid** was incorporated into the expected glycosidic linkage was  
obtained by demonstrating sensitivity of the label to digestion with  
**recombinant** Vibrio cholerae sialidase. The predicted absence of  
the biosynthetic genes for **sialic acid** synthesis  
suggests, as we have shown previously for H. influenzae, that P. multocida  
acquires free **sialic acid** from its host and then makes  
a metabolic decision between catabolism or activation for cell surface  
sialylation. That P. multocida may synthesize two **CMP-**  
**sialic acid synthetases**, one of which is  
encoded by the last gene of a **sialic acid** catabolic  
operon, suggests this HAP bacterium enjoys considerable flexibility in its  
sialometabolism, potentially accounting for its wide host range.

L17 ANSWER 9 OF 22 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2000:628244 HCAPLUS  
DOCUMENT NUMBER: 133:218534  
TITLE: **Human** glycosylation enzymes and cDNAs and  
their use in drug screening, diagnosis, and therapy  
INVENTOR(S): Coleman, Timothy A.  
PATENT ASSIGNEE(S): Human Genome Sciences, Inc., USA  
SOURCE: PCT Int. Appl., 115 pp.  
CODEN: PIXXD2  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
-----	----	-----	-----	-----
WO 2000052136	A2	20000908	WO 2000-US5325	20000301
WO 2000052136	A3	20001228		
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
AU 2000033884	A5	20000921	AU 2000-33884	20000301
EP 1159406	A2	20011205	EP 2000-912096	20000301
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
US 6333182	B1	20011225	US 2000-516143	20000301
JP 2002537796	T2	20021112	JP 2000-602748	20000301
US 2002137175	A1	20020926	US 2001-984205	20011029
US 6783971	B2	20040831		
US 2004142442	A1	20040722	US 2004-759277	20040120
PRIORITY APPLN. INFO.:			US 1999-122409P	P 19990302
			US 2000-516143	A3 20000301
			WO 2000-US5325	W 20000301
			US 2001-984205	A3 20011029

AB The present invention relates to novel **human** glycosylation enzymes and isolated nucleic acids containing the coding regions of the genes encoding such enzymes. Also provided are vectors, host cells, antibodies, and **recombinant** methods for producing **human** glycosylation enzymes. The invention further relates to diagnostic and therapeutic methods useful for diagnosing and treating disorders related to these novel **human** glycosylation enzyme polypeptides. Thus, a **human** cDNA encoding a protein with significant sequence homol. to mouse **CMP N-acetylneuraminic acid synthetase** was **cloned** and sequenced. This gene was **expressed** primarily in colon tissue. Another **human** cDNA encoded a protein with significant sequence homol. to *C. jejuni* cytidine **sialic acid synthetase**. A third **human** cDNA encoding a protein with significant sequence homol. to *E. coli* N-acetylneuraminic acid aldolase was **cloned** and sequenced. This gene was **expressed** primarily in immune cells and tissues such as primary dendritic cells, monocytes, and bone marrow.

L17 ANSWER 10 OF 22 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2000:628243 HCAPLUS

DOCUMENT NUMBER: 133:233546

TITLE: Engineering of intracellular sialylation pathways for sialylated glycoprotein production

INVENTOR(S): Betenbaugh, Michael J.; Lawrence, Shawn; Lee, Yuan C.; Jarvis, Don; Coleman, Timothy A.

PATENT ASSIGNEE(S): Human Genome Sciences, Inc., USA; Johns Hopkins University; University of Wyoming

SOURCE: PCT Int. Appl., 145 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
-----	----	-----	-----	-----
WO 2000052135	A2	20000908	WO 2000-US5313	20000301
WO 2000052135	A3	20040108		

W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU,  
 CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL,  
 IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA,  
 MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI,  
 SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM,  
 AZ, BY, KG, KZ, MD, RU, TJ, TM  
 RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE,  
 DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF,  
 CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

AU 2000035083	A5	20000921	AU 2000-35083	20000301
JP 2003524395	T2	20030819	JP 2000-602747	20000301
EP 1399538	A2	20040324	EP 2000-913684	20000301

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,  
 IE, FI, CY

US 2002142386	A1	20021003	US 2001-930440	20010816
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PRIORITY APPLN. INFO.:

US 1999-122582P	P	19990302
US 1999-169624P	P	19991208
WO 2000-US5313	W	20000301
US 2000-227579P	P	20000825

AB Methods for manipulating carbohydrate processing pathways in cells of interest are provided. Methods are directed at manipulating multiple pathways involved with the sialylation reaction by using **recombinant** DNA technol. and substrate feeding approaches to enable the production of sialylated glycoproteins in cells of interest. These carbohydrate engineering efforts encompass the implementation of new carbohydrate bioassays, the examination of a selection of insect cell lines and the use of bioinformatics to identify gene sequences for critical processing enzymes. The compns. comprise cells of interest producing sialylated glycoproteins. The methods and compns. are useful for heterologous **expression** of glycoproteins. Thus, the cDNA for a **human sialic acid 9-phosphate synthetase** which produces phosphorylated KDN and Neu5Ac from ManNAc-6-P and Man-6-P was **cloned** and sequenced. Sf9 cells infected with a baculovirus encoding this enzymes produced enhanced levels of **sialic acids** when the culture medium was supplemented with ManNAc.

L17 ANSWER 11 OF 22 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2001:374744 HCAPLUS

DOCUMENT NUMBER: 135:151270

TITLE: Sialylation of lipooligosaccharide cores affects immunogenicity and serum resistance of Campylobacter jejuni

AUTHOR(S): Guerry, Patricia; Ewing, Cheryl P.; Hickey, Thomas E.; Prendergast, Martina M.; Moran, Anthony P.

CORPORATE SOURCE: Enteric Diseases Department, Naval Medical Research Center, Silver Spring, MD, 20910, USA

SOURCE: Infection and Immunity (2000), 68(12), 6656-6662  
 CODEN: INFIBR; ISSN: 0019-9567

PUBLISHER: American Society for Microbiology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Three genes involved in biosynthesis of the lipooligosaccharide (LOS) core of Campylobacter jejuni MSC57360, the type strain of the HS:1 serotype, whose structure mimics GM2 ganglioside, have been **cloned** and characterized. Mutation of genes encoding proteins with homol. to a sialyl transferase (cstII) and a putative N-acetylmannosamine **synthetase** (neuCl), part of the biosynthetic pathway of N-acetylneuraminic acid (NeuNAc), have identical phenotypes. The LOS cores of these mutants display identical changes in electrophoretic mobility, loss of reactivity with cholera toxin (CT), and enhanced immunoreactivity with a hyperimmune polyclonal antiserum generated against whole cells of C. jejuni MSC57360. Loss of **sialic acid** in the core of the neuCl mutant was confirmed by fast atom bombardment mass spectrometry. Mutation of a gene encoding a putative

$\beta$ -1,4-N-acetylgalactosaminyltransferase (Cgt) resulted in LOS cores intermediate in electrophoretic mobility between that of wild type and the mutants lacking NeuNAc, loss of reactivity with CT, and a reduced immunoreactivity with hyperimmune antiserum. Chemical analyses confirmed the loss of N-acetylgalactosamine (GalNAc) and the presence of NeuNAc in the cgt mutant. These data suggest that the Cgt enzyme is capable of transferring GalNAc to an acceptor with or without NeuNAc and that the Cst enzyme is capable of transferring NeuNAc to an acceptor with or without GalNAc. A mutant with a nonsialylated LOS core is more sensitive to the bactericidal effects of human sera than the wild type or the mutant lacking GalNAc.

REFERENCE COUNT: 53 THERE ARE 53 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L17 ANSWER 12 OF 22 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation.  
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ACCESSION NUMBER: 1999:149430 SCISEARCH

THE GENUINE ARTICLE: 166KQ

TITLE: Haemophilus ducreyi produces a novel sialyltransferase - Identification of the sialyltransferase gene and construction of mutants deficient in the production of the sialic acid-containing glycoform of the lipooligosaccharide

AUTHOR: Bozue J A; Tullius M V; Wang J; Gibson B W; Munson R S (Reprint)

CORPORATE SOURCE: CHILDRENS HOSP RES FDN, 700 CHILDRENS DR, ROOM W402, COLUMBUS, OH 43205 (Reprint); CHILDRENS HOSP RES FDN, COLUMBUS, OH 43205; OHIO STATE UNIV, DEPT PEDIAT, COLUMBUS, OH 43205; OHIO STATE UNIV, DEPT MED MICROBIOL, COLUMBUS, OH 43205; UNIV CALIF SAN FRANCISCO, DEPT PHARMACEUT CHEM, SAN FRANCISCO, CA 94143

COUNTRY OF AUTHOR: USA

SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (12 FEB 1999) Vol. 274, No. 7, pp. 4106-4114.  
Publisher: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814.  
ISSN: 0021-9258.

DOCUMENT TYPE: Article; Journal

FILE SEGMENT: LIFE

LANGUAGE: English

REFERENCE COUNT: 76

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Haemophilus ducreyi, the cause of the sexually transmitted disease chancroid produces a lipooligosaccharide (LOS) containing a terminal sialyl N-acetyllactosamine trisaccharide. Previously, we reported the identification and characterization of the N-acetylneuraminic acid cytidylsynthetase gene (neuA), Forty-nine base pairs downstream of the synthetase gene is an open reading frame (ORF) encoding a protein with a predicted molecular weight of 34,646. This protein has weak homology to the polysialyltransferase of Escherichia coli K92. Downstream of this ORF is the gene encoding the H, ducreyi homologue of the Salmonella typhimurium rmlB gene. Mutations were constructed in the neuA gene and the gene encoding the second ORF by insertion of an Omega kanamycin cassette, and isogenic strains were constructed. LOS was isolated from each strain and characterized by SDS-polyacrylamide gel electrophoresis, carbohydrate, and mass spectrometric analysis. LOS isolated from strains containing a mutation in neuA or in the second ORF, designated Ist, lacked the sialic acid-containing glycoform. Complementation studies were performed. The neuA gene and the ist gene were each cloned into the shuttle vector pLS88 after polymerase chain reaction amplification. Complementation of the mutation in the ist gene was observed, but we were unable to complement the neuA mutation. Since it is possible that transcription of the neuA gene and the Ist gene were coupled, we constructed a nonpolar mutation in the neuA

gene, in this construct, the neuA mutation was complemented, suggesting transcriptional coupling of the neuA gene and the ist gene, Sialyltransferase activity was detected by incorporation of C-14-labeled NeuAc from **CMP**-NeuAc into trichloroacetic acid-precipitable material when the Ist gene was overexpressed in the nonpolar neuA mutant. We conclude that the Ist gene encodes the H, ducreyi sialyltransferase, Since the Ist gene product has little, if any, structural relationship to other sialyltransferases, this protein represents a new class of sialyltransferase.

L17 ANSWER 13 OF 22 MEDLINE on STN DUPLICATE 3

ACCESSION NUMBER: 2000084724 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 10619706  
TITLE: Combinatorial PCR approach to homology-based  
**cloning: cloning and expression**  
of mouse and **human** GM3-synthase.  
AUTHOR: Kapitonov D; Bieberich E; Yu R K  
CORPORATE SOURCE: Department of Biochemistry and Molecular Biophysics,  
Medical College of Virginia, Virginia Commonwealth  
University Richmond, 23298-0614, USA.  
CONTRACT NUMBER: NS11853 (NINDS)  
SOURCE: Glycoconjugate journal, (1999 Jul) 16 (7) 337-50.  
Journal code: 8603310. ISSN: 0282-0080.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 200002  
ENTRY DATE: Entered STN: 20000229  
Last Updated on STN: 20000229  
Entered Medline: 20000214

AB GM3-synthase, also known as sialyltransferase I (ST-I), catalyzes the transfer of a **sialic acid** residue from **CMP-sialic acid** onto lactosylceramide to form ganglioside GM3. In order to **clone** this enzyme, as well as other sialyltransferases, we developed an approach that we termed combinatorial PCR. In this approach, degenerate primers were designed on the basis of conserved sequence motifs of the ST3 family of sialyltransferases (STs). The nucleotide sequence of the primers was varied to cover all amino acid variations occurring in each motif. In addition, in some primers the sequence was varied to cover possible homologous substitutions that are absent in the available motifs. A panel of cDNA from 12 mouse and 8 **human** tissues was used to enable **cloning** of tissue- and stage-specific sialyltransferases. Using this approach, the fragments of 11 new putative sialyltransferases were isolated and sequenced so far. Analysis of the **expression** pattern of a particular sialyltransferase across the panel of cDNA from the different tissues provided information about the tissue specificity of ST **expression**. We chose two new ubiquitously **expressed human** and mouse STs to **clone** full-length copies and to assay for GM3-synthase activity. One of the STs, which exhibited the highest homology to ST3 Gal III, showed activity toward lactosylceramide (LacCer) and was termed ST3 Gal V according to the suggested nomenclature [1]. The other ubiquitously **expressed** sialyltransferase was termed ST3Gal VI. All isolated sialyltransferases were screened for alternatively spliced forms (ASF). Such forms were found for both **human** ST3Gal V and ST3Gal VI in **human** fetal brain cDNA library. The detailed **cloning** strategy, functional assay, and full length cDNA and protein sequences of GM3 synthase (ST3Gal V, or ST-I) are presented.

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ACCESSION NUMBER: 96:508623 SCISEARCH

THE GENUINE ARTICLE: UV299  
 TITLE: PURIFICATION, **CLONING**, AND **EXPRESSION**  
 OF A CYTIDINE 5'-MONOPHOSPHATE N-ACETYLNEURAMINIC ACID  
**SYNTHETASE** FROM HAEMOPHILUS-DUCREYI  
 AUTHOR: TULLIUS M V; MUNSON R S; WANG J; GIBSON B W (Reprint)  
 CORPORATE SOURCE: UNIV CALIF SAN FRANCISCO, SCH PHARM, DEPT PHARMACEUT CHEM,  
 926-S, 513 PARNASSUS AVE, SAN FRANCISCO, CA, 94143  
 (Reprint); UNIV CALIF SAN FRANCISCO, SCH PHARM, DEPT  
 PHARMACEUT CHEM, SAN FRANCISCO, CA, 94143; OHIO STATE  
 UNIV, CHILDRENS HOSP, RES FDN, COLUMBUS, OH, 43205; OHIO  
 STATE UNIV, DEPT PEDIAT, COLUMBUS, OH, 43205; OHIO STATE  
 UNIV, DEPT MED MICROBIOL & IMMUNOL, COLUMBUS, OH, 43205  
 COUNTRY OF AUTHOR: USA  
 SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (28 JUN 1996) Vol. 271,  
 No. 26, pp. 15373-15380.  
 ISSN: 0021-9258.  
 DOCUMENT TYPE: Article; Journal  
 FILE SEGMENT: LIFE  
 LANGUAGE: ENGLISH  
 REFERENCE COUNT: 60

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB An N-acetylneuraminic acid cytidyltransferase (EC 2.7.7.43) (**CMP-NeuAc synthetase**) was isolated from a Haemophilus ducreyi strain 35000 cell lysate and partially characterized. The enzyme catalyzes the reaction of CTP and NeuAc to form **CMP-NeuAc**, which is the nucleotide sugar donor used by sialyltransferases. Previous studies have shown that the outer membrane lipooligosaccharides of H. ducreyi contain terminal **sialic acid** attached to N-acetylglucosamine and that this modification is likely important to its pathogenesis. Therefore, to investigate the role of **sialic acid** in a. ducreyi pathogenesis, the gene encoding the **CMP-NeuAc synthetase** was **cloned** using degenerate oligonucleotide probes derived from NH2-terminal sequence data, and the nucleotide sequence was determined. The derived amino acid sequence of the **CMP-NeuAc synthetase** gene has homology to other **CMP-NeuAc synthetases** and to a lesser extent to **CMP-2-keto-3-deoxy-D-manno-octulosonic acid synthetases**. The gene was **cloned** into a T7 **expression** vector, the protein **expressed** in Escherichia coli, and purified to apparent homogeneity by anion exchange, Green 19 dye, and hydrophobic interaction chromatography. The final step yielded 20 mg of pure protein/liter of culture. The protein has a predicted molecular mass of 25440.6 Pa, which was confirmed by electrospray mass spectrometry ( $M(\text{expt}) = 25439.9 \pm 1.4$  Pa). The enzyme appears to exist as a dimer by size exclusion chromatography. In contrast to other bacterial **CMP-NeuAc synthetases**, the H. ducreyi enzyme exhibited a different substrate specificity, being capable of also using N-glycolylneuraminic acid as a substrate.

L17 ANSWER 15 OF 22 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation.  
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ACCESSION NUMBER: 96:752059 SCISEARCH  
 THE GENUINE ARTICLE: VL560  
 TITLE: THE BIOCHEMISTRY AND GENETICS OF CAPSULAR POLYSACCHARIDE  
 PRODUCTION IN BACTERIA  
 AUTHOR: ROBERTS I S (Reprint)  
 CORPORATE SOURCE: UNIV MANCHESTER, SCH BIOL SCI, MANCHESTER M13 9PT, LANCS,  
 ENGLAND (Reprint)  
 COUNTRY OF AUTHOR: ENGLAND  
 SOURCE: ANNUAL REVIEW OF MICROBIOLOGY, (1996) Vol. 50, pp. 285-315  
 ISSN: 0066-4227.  
 DOCUMENT TYPE: General Review; Journal  
 FILE SEGMENT: LIFE

LANGUAGE: ENGLISH

REFERENCE COUNT: 143

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Bacterial polysaccharides are usually associated with the outer surface of the bacterium. They can form an amorphous layer of extracellular polysaccharide (EPS) surrounding the cell that may be further organized into a distinct structure termed a capsule. Additional polysaccharide molecules such as lipopolysaccharide (LPS) or lipooligosaccharide (LOS) may also decorate the cell surface. Polysaccharide capsules may mediate a number of biological processes, including invasive infections of human beings. Discussed here are the genetics and biochemistry of selected bacterial capsular polysaccharides and the basis of capsule diversity but not the genetics and biochemistry of LPS biosynthesis (for reviews see 100, 140).

L17 ANSWER 16 OF 22 MEDLINE on STN

ACCESSION NUMBER: 96004600 MEDLINE

DOCUMENT NUMBER: PubMed ID: 7567994

TITLE: Regulation of glycolipid synthesis in HL-60 cells by antisense oligodeoxynucleotides to glycosyltransferase sequences: effect on cellular differentiation.

AUTHOR: Zeng G; Ariga T; Gu X B; Yu R K

CORPORATE SOURCE: Department of Biochemistry and Molecular Biophysics, Medical College of Virginia, Virginia Commonwealth University, Richmond 23298-0614, USA.

CONTRACT NUMBER: NS-11853 (NINDS)

SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (1995 Sep 12) 92 (19) 8670-4. Journal code: 7505876. ISSN: 0027-8424.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199510

ENTRY DATE: Entered STN: 19951227

Last Updated on STN: 19980206

Entered Medline: 19951023

AB Treatment of the human promyelocytic leukemia cell line HL-60 with antisense oligodeoxynucleotides to UDP-N-acetylgalactosamine:beta-1,4-N-acetylgalactosaminyl-transferase (GM2-synthase; EC 2.4.1.92) and **CMP-sialic acid**:alpha-2,8-sialyltransferase (GD3-synthase; EC 2.4.99.8) sequences effectively down-regulated the synthesis of more complex gangliosides in the ganglioside synthetic pathways after GM3, resulting in a remarkable increase in endogenous GM3 with concomitant decreases in more complex gangliosides. The treated cells underwent monocytic differentiation as judged by morphological changes, adherent ability, and nitroblue tetrazolium staining. These data provide evidence that the increased endogenous ganglioside GM3 may play an important role in regulating cellular differentiation and that the antisense DNA technique proves to be a powerful tool in manipulating glycolipid synthesis in the cell.

L17 ANSWER 17 OF 22 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation.  
on STN

ACCESSION NUMBER: 95:52787 SCISEARCH

THE GENUINE ARTICLE: QA451

TITLE: SEQUENTIAL-CHANGES IN GLYCOLIPID **EXPRESSION**  
DURING **HUMAN** B-CELL DIFFERENTIATION - ENZYMATIC  
BASES

AUTHOR: TAGA S; TETAUD C; MANGENEY M; TURSZ T; WIELS J (Reprint)

CORPORATE SOURCE: INST GUSTAVE ROUSSY, BIOL TUMEURS HUMAINES LAB, CNRS, URA 1156, RUE CAMILLE DESMOULINS, F-94805 VILLEJUIF, FRANCE  
(Reprint); INST GUSTAVE ROUSSY, BIOL TUMEURS HUMAINES LAB, CNRS, URA 1156, F-94805 VILLEJUIF, FRANCE

COUNTRY OF AUTHOR: FRANCE  
 SOURCE: BIOCHIMICA ET BIOPHYSICA ACTA-LIPIDS AND LIPID METABOLISM,  
 (03 JAN 1995) Vol. 1254, No. 1, pp. 56-65.  
 ISSN: 0005-2760.  
 DOCUMENT TYPE: Article; Journal  
 FILE SEGMENT: LIFE  
 LANGUAGE: ENGLISH  
 REFERENCE COUNT: 44

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB We have previously reported that **human** B cell differentiation is accompanied by sequential changes in glycosphingolipid **expression**. Pre-B cells contain lacto-series type II chain-based glycolipids and GM3 ganglioside; mature/activated B cells do not synthesize lacto-series compounds but **express** GM3 and globo-series glycolipids (Gb3 and Gb4); terminally differentiated B cells, in addition to these compounds, also contain GM2 ganglioside. At the cell surface, Gb3, Gb3 and GM2 constitute stage-specific antigens. To elucidate the biosynthetic mechanism leading to these modifications we have compared activities of the glycosyltransferases involved in the core structure assembly and the first elongation steps of neo-facto, ganglio- and globo-series glycolipids. These glycosyltransferase activities have been measured in B cell lines and normal B lymphocytes at various stages of differentiation. We first determined the optimal requirements of the four glycosyltransferases which synthesize Lc3, GM3, Gb4 and GM2 glycolipids in B lymphocytes and then tested these enzymes and the Gb3 **synthetase** in the selected B cells. The following results were obtained: beta 1 --> 3 N-Acetylglucosaminyltransferase (Lc3 **synthetase**) has a high activity in pro- and pre-B cells whereas it is undetectable in more differentiated cells; alpha 2 --> 3 sialyltransferase (GM3 **synthetase**) is activated from the pre-B cell stage to the terminally differentiated myeloma cells; alpha 1 --> 4 galactosyltransferase (Gb3 **synthetase**) is only detected in cells representing the late stages of B cell differentiation; beta 1 --> 3 N-Acetylgalactosaminyltransferase (Gb4 **synthetase**) is only found in some lymphoblastoid cell lines, representative of activated B cells whereas the beta 1 --> 4 N-Acetylgalactosaminyltransferase (GM2 **synthetase**) has a high activity in these lymphoblastoid cell lines and in terminally differentiated myeloma cells. These results suggest that the sequential shifts in the three major glycosphingolipid series observed during B cell differentiation are mostly due to sequential activations of the corresponding glycosyltransferases.

L17 ANSWER 18 OF 22 MEDLINE on STN DUPLICATE 4  
 ACCESSION NUMBER: 95162590 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 7858974  
 TITLE: Combined chemical and enzymatic synthesis of the sialylated non reducing terminal sequence of GM1b glycolylated ganglioside, a potential **human** tumor marker.  
 AUTHOR: Lubineau A; Auge C; Gautheron-Le Narvor C; Ginet J C  
 CORPORATE SOURCE: Institut de Chimie Moleculaire d'Orsay, URA CNRS 462, Universite Paris-Sud, Orsay, France.  
 SOURCE: Bioorganic & medicinal chemistry, (1994 Jul) 2 (7) 669-74. Journal code: 9413298. ISSN: 0968-0896.  
 PUB. COUNTRY: ENGLAND: United Kingdom  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 199503  
 ENTRY DATE: Entered STN: 19950404  
 Last Updated on STN: 19980206  
 Entered Medline: 19950320

AB N-Glycolylglucosamine 8 was synthesized in 4 steps from anisal glucosamine, via the new crystalline monochloroacetyl derivatives 3, 4 and 7. N-Glycolylneuraminic acid 10 was prepared in 59% yield starting from

pyruvate and a mixture of 8 and its manno epimer 9 in a 2:3 ratio, with immobilized **sialic acid** aldolase. Neu5Gc 10 was converted into **CMP-NeuGc** 11 in the presence of immobilized calf brain **CMP-sialate synthetase**. Finally 11 was used as a donor in the transfer to the acceptor beta-D-Gal-(1-3)-beta-D-GalNAc-OBn 12 catalyzed by a preparation of porcine liver (2-3)-alpha-sialyltransferase, roughly purified by a chromatography on Cibacron Blue-agarose. alpha-Neu5Gc-(2-3)-beta-D-Gal-(1-3)-beta-D-GalNAc-OBn 13 isolated in 56% yield was deprotected to give the non-reducing terminal sequence of GM1b glycolylated ganglioside, which might be **expressed** in **human** tumors.

L17 ANSWER 19 OF 22 MEDLINE on STN DUPLICATE 5  
 ACCESSION NUMBER: 92112296 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 1309720  
 TITLE: Identification of a genetic locus essential for capsule sialylation in type III group B streptococci.  
 AUTHOR: Wessels M R; Haft R F; Heggen L M; Rubens C E  
 CORPORATE SOURCE: Channing Laboratory, Department of Medicine, Brigham and Women's Hospital, Boston, Massachusetts.  
 CONTRACT NUMBER: AI07061 (NIAID)  
 AI22498 (NIAID)  
 AI28040 (NIAID)  
 SOURCE: Infection and immunity, (1992 Feb) 60 (2) 392-400.  
 Journal code: 0246127. ISSN: 0019-9567.  
 PUB. COUNTRY: United States  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 199202  
 ENTRY DATE: Entered STN: 19920308  
 Last Updated on STN: 19990129  
 Entered Medline: 19920218

AB The type III capsular polysaccharide of group B streptococci (GBS) consists of a linear backbone with short side chains ending in residues of N-acetylneuraminic acid, or **sialic acid**. The presence of **sialic acid** on the surface of the organism inhibits activation of the alternative pathway of complement and is thought to be an important element in the virulence function of the capsule. We showed previously that a mutant strain of GBS that **expressed** a **sialic acid**-deficient, or asialo, form of the type III polysaccharide was avirulent, supporting a virulence function for capsular **sialic acid**. We now report the derivation of an asialo capsule mutant from a highly encapsulated wild-type strain of type III GBS, strain COH1, by insertional mutagenesis with transposon Tn916 delta E. In contrast to the wild-type strain, the asialo mutant strain COH1-11 was sensitive to phagocytic killing by **human** leukocytes in vitro and was relatively avirulent in a neonatal rat model of GBS infection. The asialo mutant accumulated free intracellular **sialic acid**, suggesting a defect subsequent to **sialic acid** synthesis in the biosynthetic pathway leading to capsule sialylation. The specific biosynthetic defect in mutant strain COH1-11 was found to be in the activation of free **sialic acid** to **CMP-sialic acid**: **CMP-sialic acid synthetase** activity was present in the wild-type strain COH1 but was not detected in the asialo mutant strain COH1-11. One of the two transposon insertions in the asialo mutant COH1-11 mapped to the same chromosomal location as one of the two Tn916 insertions in the previously reported asialo mutant COH31-21, identifying this site as a genetic locus necessary for **expression** of **CMP-sialic acid synthetase** activity. These studies demonstrate that the enzymatic synthesis of **CMP-sialic acid** by GBS is an essential step in sialylation of the type III capsular polysaccharide.

	L #	Hits	Search Text
1	L1	1	6783971.pn.
2	L2	2444	"30 contiguous"
3	L3	1	l1 and l2
4	L4	428	fragment same "50 contiguous"
5	L5	0	l1 and l4
6	L6	2759	"50 contiguous"
7	L7	1	l1 and l6
8	L8	12362	synthetase\$2
9	L9	32743	"CMP"
10	L10	4051	"sialic acid"
11	L11	88	l8 same l10
12	L12	79	l9 same l11
13	L13	664337	clon\$3 or express\$3 or recombinant

	L #	Hits	Search Text
14	L14	36	l12 same l13
15	L15	43679 2	human
16	L17	31778	glycosylat\$3
17	L18	0	l14 same l17
18	L16	8	l14 same l15
19	L19	22272	COLEMAN BETENBAUGH
20	L20	6	l12 and l19

	Issue Date	Pages	Document ID	Title
1	20040916	85	US 20040180406 A1	Nucleic acids encoding sialyltransferases from <i>C. jejuni</i>
2	20040812	31	US 20040156837 A1	<i>Haemophilus influenzae</i> sialyltransferase and methods of use thereof
3	20040722	39	US 20040142442 A1	Human glycosylation enzymes
4	20040226	259	US 20040038207 A1	Gene expression in bladder tumors
5	20040212	570	US 20040029114 A1	Methods of diagnosis of breast cancer, compositions and methods of screening for modulators of breast cancer
6	20040129	84	US 20040018522 A1	Identification of dysregulated genes in patients with multiple sclerosis
7	20040115	484	US 20040009479 A1	Methods and compositions for diagnosing or monitoring auto immune and chronic inflammatory diseases
8	20031002	31	US 20030186414 A1	Nucleic acid that encodes a fusion protein
9	20030925	26	US 20030180928 A1	Fusion protein comprising a UDP-Galnac 4' epimerase and a galnac transferase

	Issue Date	Pages	Document ID	Title
10	20030821	84	US 20030157658 A1	Polypeptides having beta-1,4-GalNAc transferase activity
11	20030821	84	US 20030157657 A1	Polypeptides having beta-1,3-galactosyl transferase activity
12	20030821	85	US 20030157656 A1	Nucleic acids encoding beta-1,4-GalNAc transferase
13	20030821	84	US 20030157655 A1	Nucleic acids encoding polypeptides with beta-1,3-galactosyl transferase activity
14	20030814	278	US 20030154032 A1	Methods and compositions for diagnosing and treating rheumatoid arthritis
15	20030807	84	US 20030148459 A1	Polypeptides having sialyltransferase activity
16	20030501	78	US 20030082511 A1	Identification of modulatory molecules using inducible promoters
17	20021017	48	US 20020150968 A1	Glycoconjugate and sugar nucleotide synthesis using solid supports
18	20021003	88	US 20020142386 A1	Engineering intracellular sialylation pathways
19	20020926		US 20020137175 A1	Human glycosylation enzymes

	Issue Date	Pages	Document ID	Title
20	20020919		US 20020132320 A1	Glycoconjugate synthesis using a pathway-engineered organism
21	20020509		US 20020055168 A1	Streptococcus suis vaccines and diagnostic tests
22	20020411		US 20020042369 A1	Campylobacter glycosyltransferases for biosynthesis of gangliosides and ganglioside mimics
23	20020321		US 20020034805 A1	FUSION PROTEINS FOR USE IN ENZYMATIC SYNTHESIS OF OLIGOSACCHARIDES
24	20020103		US 20020001831 A1	Low cost manufacture of oligosaccharides
25	20040831	46	US 6783971 B2	Human glycosylation enzymes
26	20040420		US 6723545 B2	Polypeptides having .beta.-1,4-GalNAc transferase activity
27	20040302		US 6699705 B2	Campylobacter glycosyltransferases for biosynthesis of gangliosides and ganglioside mimics
28	20030107		US 6503744 B1	Campylobacter glycosyltransferases for biosynthesis of gangliosides and ganglioside mimics
29	20020611		US 6403306 B1	Serogroup-specific nucleotide sequences in the molecular typing of bacterial isolates and the preparation of vaccines thereto

	Issue Date	Pages	Document ID	Title
30	20011225		US 6333182 B1	Human glycosylation enzymes
31	20010102		US 6168934 B1	Oligosaccharide enzyme substrates and inhibitors: methods and compositions
32	20000912		US 6117651 A	Expression vectors
33	19980602		US 5759823 A	Oligosaccharide enzyme substrates and inhibitors: methods and compositions
34	19970114		US 5593887 A	Oligosaccharide enzyme substrates and inhibitors: methods and compositions
35	19951024		US 5461143 A	Oligosaccharide enzyme substrates and inhibitors: methods and compositions

	Issue Date	Pages	Document ID	Title
36	19940111		US 5278299 A	Method and composition for synthesizing sialylated glycosyl compounds

	Issue Date	Pages	Document ID	Title
1	20040722	39	US 20040142442 A1	Human glycosylation enzymes
2	20040226	259	US 20040038207 A1	Gene expression in bladder tumors
3	20040212	570	US 20040029114 A1	Methods of diagnosis of breast cancer, compositions and methods of screening for modulators of breast cancer
4	20030814	278	US 20030154032 A1	Methods and compositions for diagnosing and treating rheumatoid arthritis
5	20021003	88	US 20020142386 A1	Engineering intracellular sialylation pathways
6	20020926	46	US 20020137175 A1	Human glycosylation enzymes
7	20040831	46	US 6783971 B2	Human glycosylation enzymes
8	20011225	47	US 6333182 B1	Human glycosylation enzymes

	Issue Date	Pages	Document ID	Title
1	20040722	39	US 20040142442 A1	Human glycosylation enzymes
2	20021003	88	US 20020142386 A1	Engineering intracellular sialylation pathways
3	20020926	46	US 20020137175 A1	Human glycosylation enzymes
4	20020530	73	US 20020065404 A1	Cytidine monophosphate-sialic acid transporter, and hexosaminidase polynucleotides and polypeptides, and uses based thereon.
5	20040831	46	US 6783971 B2	Human glycosylation enzymes
6	20011225	47	US 6333182 B1	Human glycosylation enzymes

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=> s "sialic acid synthetase?"

L1 195 "SIALIC ACID SYNTHETASE?"

=> s "CMP"

L2 21033 "CMP"

=> s l1 and l2

L3 180 L1 AND L2

=> s human and l3

L4 27 HUMAN AND L3

=> s clon? or express? or recombinant

5 FILES SEARCHED...

L5 6727337 CLON? OR EXPRESS? OR RECOMBINANT

=> s l4 and l5

L6 14 L4 AND L5

=> dup rem l6

PROCESSING COMPLETED FOR L6

L7 10 DUP REM L6 (4 DUPLICATES REMOVED)

=> d 1-10 ibib ab

L7 ANSWER 1 OF 10 BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN

ACCESSION NUMBER: 2004-18268 BIOTECHDS

TITLE: Producing glycoprotein with animal type sugar chain,  
comprises introducing gene encoding enzyme that adds sialic  
acid to non-reducing terminal of sugar chain, and gene of  
heterologous protein, into plant cell, cultivating plant cell

;

transgenic plant construction via bacterium-mediated  
transformation for use in protein production

AUTHOR: FUJIYAMA K; SEKI T

PATENT ASSIGNEE: FUJIYAMA K; SEKI T  
PATENT INFO: WO 2004063370 29 Jul 2004  
APPLICATION INFO: WO 2004-P 264 15 Jan 2004  
PRIORITY INFO: JP 2003-7687 15 Jan 2003; JP 2003-7687 15 Jan 2003  
DOCUMENT TYPE: Patent  
LANGUAGE: Japanese  
OTHER SOURCE: WPI: 2004-561900 [54]

AB DERWENT ABSTRACT:

NOVELTY - Producing (M1) glycoprotein (I) having animal type sugar chain, involves introducing a gene encoding the enzyme that can add sialic acid to the non-reducing terminal of sugar chain, and the gene of heterologous protein, into the plant cell, cultivating the transformed plant cell, and recovering the culture solution of the plant cell.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) plant cell (II) transformed with the gene that codes

**sialic acid synthetase, CMP-**

**sialic acid synthetase and/or CMP**

-sialic acid transporter exhibiting saccharide addition mechanism (adding sialic acid to the non-reducing terminal of the sugar chain of glycoprotein), where (II) can take in the precursor of sialic acid or sialic acid, and has a vesicle that allows the uptake of sialic acid; and (2) plant body regenerate from (II).

BIOTECHNOLOGY - Preferred Method: In (M1), the glycoprotein having animal type sugar chain contains a core sugar chain and the external sugar chain, where the core sugar chain essentially comprises several numbers of mannose and acetylglucosamine, while the external sugar chain has a terminal sugar chain moiety containing a non-reducing end galactose. The external sugar chain is the linear or mono, tri or tetra branched sugar chain.

USE - (M1) is useful for producing glycoprotein having animal type sugar chain (claimed).

EXAMPLE - The DNA of the Escherichia coli was used as the template, PCR was performed using the primers having the sequences such as 5'-tttagctcgagacaatgagtaatatatat-3' and 5'-tttttctcgagttattattccccctgatttttaaattc-3', the obtained PCR product was digested by XhoI and SalI restriction enzymes, and the neuB fragment was obtained. The DNA of the Nicotiana tabacum cv SRI was used as the template, PCR was performed using the primers having the sequences such as 5'-tttaagtcgacacgatgagagg-3' and 5'-aatcgtcgacccttaactgtc-3', the obtained PCR product was digested by restriction enzymes. The obtained neuB fragment, and the **CMP-sialic acid transporter (CST)** gene were connected, and the target CTS-neuB gene was obtained. The obtained CTS-neuB gene was introduced into the plasmid pBI221, and the vector pBI121-CTS-neuB was obtained. The XhoI and SpeI restriction enzyme site was introduced into the vector pBI121-CTS-neuB by PCR amplification using specific primers, and the plasmid containing the **expression** cassette of CTS-neuB gene was obtained. The cDNA of the **human** kidney was used as the template, PCR was performed using the primers having the sequences such as 5'-gttactagtagtgactcggtggagaagggcgccacctccgtcctcaaccgcggggcgaccgtccc-3' and 5'-tgggagctcctatatttggcatgaattatt-3', the obtained PCR product was introduced into the plasmid pBI221. The obtained **expression** cassette was then introduced into pGPTV-HPT, and the pGPTV-HPT-hCSS was obtained. The XhoI and SpeI restriction enzyme site was introduced into the vector pGPTV-HPT-hCSS by PCR amplification using specific primers, and the plasmid containing the **expression** cassette of HPT-hCSS gene was obtained. The plasmid containing the **expression** cassette of HPT-hCST was also obtained. The plasmids containing the **expression** cassettes of CST-neuB gene and the HPT-hCST gene were digested by XhoI and SpeI enzymes, and both the XhoI/SpeI fragments were introduced into pGEM-T Easy vector, and the plasmid pGEM-T-hCST-CTS-neuB was obtained. To the plasmid pGEM-T-hCST-CTS-neuB gene, the **expression** cassette of HPT-hCSS was introduced, and the plasmid pGPTV-HPT-hCSS-hCST-CTS-neuB was obtained. The pGPTV-HPT-hCSS-hCST-CTS-neuB was introduced into

Agrobacterium tumefaciens LBA4404. The transformed LBA4404 was allowed to infect the cell of tobacco. The infected tobacco cell was cultivated for 7 days, the DNA was isolated from the plant cell, and the presence of the **CMP-sialic acid synthetase** (CSS) and **CMP-sialic acid transporter** (CST) gene was detected. The results showed the presence of CSS and CST genes in the transformed plant cell. Thus the plant cell containing CST and CSS in its genome was obtained. (88 pages)

L7 ANSWER 2 OF 10 MEDLINE on STN  
 ACCESSION NUMBER: 2004336041 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 15238249  
 TITLE: **CMP-sialic acid synthetase** of the nucleus.  
 AUTHOR: Kean Edward L; Munster-Kuhnel Anja K; Gerardy-Schahn Rita  
 CORPORATE SOURCE: Department of Ophthalmology, Case Western Reserve University, Cleveland, OH 4410, USA.  
 SOURCE: Biochimica et biophysica acta, (2004 Jul 6) 1673 (1-2) 56-65. Ref: 73  
 Journal code: 0217513. ISSN: 0006-3002.  
 PUB. COUNTRY: Netherlands  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 General Review; (REVIEW)  
 (REVIEW, TUTORIAL)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 200408  
 ENTRY DATE: Entered STN: 20040708  
 Last Updated on STN: 20040818  
 Entered Medline: 20040817

AB Sialic acids of cell surface glycoconjugates play a pivotal role in the structure and function of animal cells and in some bacterial pathogens. The pattern of cell surface sialylation is species specific, and, in the animal, highly regulated during embryonic development. A prerequisite for the synthesis of sialylated glycoconjugates is the availability of the activated sugar-nucleotide cytidine 5'-monophosphate N-acetylneuraminic acid (**CMP-NeuAc**), which provides the substrate for sialyltransferases. Trials to purify the enzymatic activity responsible for the synthesis of **CMP-NeuAc** from different animal sources demonstrated that the major localisation of the enzyme is the cell nucleus. These earlier findings were confirmed when the murine **CMP-NeuAc synthetase** was **cloned** and the subcellular transport of **recombinant** epitope tagged forms visualised by indirect immunofluorescence. Today, the primary sequence elements that direct murine **CMP-NeuAc synthetase** into the cell nucleus are known, however, information regarding the physiological relevance of the nuclear destination is still not available. With this article, we provide a detailed review on earlier and recent findings that identified and confirmed the unusual subcellular localisation of the **CMP-NeuAc synthetase**. In addition, we take the advantage to discuss most recent developments towards understanding structure--function relations of this enzyme.

L7 ANSWER 3 OF 10 BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN  
 ACCESSION NUMBER: 2003-06688 BIOTECHDS  
 TITLE: Manipulating glycoprotein production in insect cell, involves enhancing **expression** of enzymes involved in carbohydrate processing pathway such as N-acetylglucosamine-2 epimerase or **sialic acid synthetase**;  
**recombinant** protein production via plasmid **expression** in host cell for use in diagnosis and therapy  
 AUTHOR: BETENBAUGH M J; LAWRENCE S; LEE Y C; COLEMAN T A

PATENT ASSIGNEE: BETENBAUGH M J; LAWRENCE S; LEE Y C; COLEMAN T A  
PATENT INFO: US 2002142386 3 Oct 2002  
APPLICATION INFO: US 2001-930440 16 Aug 2001  
PRIORITY INFO: US 2001-930440 16 Aug 2001; US 1999-122582 2 Mar 1999  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
OTHER SOURCE: WPI: 2003-102519 [09]

AB DERWENT ABSTRACT:

NOVELTY - Manipulating (M1) glycoprotein production in an insect cell comprising enhancing **expression** of an enzyme (E) such as N-acetylglucosamine-2 (GlcNAc-2) epimerase, one catalyzing conversion of UDP-GlcNAc to mannose (Man)NAc, **sialic acid synthetase**, aldolase, cytidine monophosphate-sialic acid (CMP-SA) synthetase or CMP-SA transporter, where the **expression** of each (E) is enhanced to above endogenous levels, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) a cell of interest (I) producing the donor substrate **CMP-SA** above endogenous levels; (2) a cell of interest (II) producing an acceptor substrate, the donor substrate **CMP-SA**, and **expressing** the enzyme sialyltransferase, where the acceptor substrate is a glycan; (3) a cell of interest (III) producing sialylated glycoprotein above endogenous levels; (4) a kit (IV) for **expression** of sialylated glycoproteins, comprising (I); (5) producing (M2) sialylated glycoproteins, by **expressing** a heterologous protein in an insect cell manipulated by M1; and (6) producing (M3) sialylated glycoprotein in a cell of interest, by determining the carbohydrate substrates in the cell, transforming the cell with enzymes to produce necessary precursor substrates, and constructing a processing pathway in the cell to produce a sialylated glycoprotein.

WIDER DISCLOSURE - Disclosed are: (A) an assay for sialylation; (B) sequence variants of the amino acid sequence or nucleotide sequence of **human aldolase**, **human CMP-SA synthetase** or SA-synthetase, and their fragments; (C) nucleic acid molecules encoding the above mentioned variant polypeptides; (D) polynucleotides having a lower degree of identity but having sufficient similarity to (E) so as to perform one or more of the same functions as (E); (E) **recombinant** vectors including isolated nucleic acid molecules encoding (E); (F) a host cell comprising the above mentioned vector and/or nucleic acid molecule; and (G) **expressing** heterologous proteins in (I), (II) or (III).

BIOTECHNOLOGY - Preferred Method: In M1, the GlcNAc-2 epimerase, the enzyme catalyzing conversion of UDP-GlcNAc to ManNAc, or **CMP-SA synthetase** is a **human** enzyme. The **expression** of (E) is enhanced by M1. The **sialic acid synthetase** has a sequence of 359 amino acids fully defined in the specification, and the aldolase has a sequence of 230 or 434 amino acids fully defined in the specification. M1 further comprises enhancing the **expression** of at least one enzyme selected from Gal T, GlcNAc TI, GlcNAc TII and sialyltransferase. M1 further comprises suppressing the activity of endogenous N-acetylglucosaminidase. In M2, the heterologous protein is a mammalian protein selected from plasminogen, transferrin, Na<sup>+</sup>, K<sup>+</sup>-ATPase or thyrotropin. In M3, the cell is a yeast, insect, fungal, plant or bacterial cell. The cell enhances the **expression** of both **sialic acid synthetase** and **CMP-SA synthetase**. Preferred Cell: In (II), the glycan is a branched glycan comprising GalGlcNAcMan by at least one branch of the glycan and the Gal is a terminal Gal. The glycan is an asparagine-linked glycan. In (III), the glycoprotein is asparagine (N)-linked, and the glycoprotein is a mammalian heterologous selected from plasminogen, transferrin, Na<sup>+</sup>, K<sup>+</sup>-ATPase, and thyrotropin. (I) **expresses** (E).

ACTIVITY - None given.

MECHANISM OF ACTION - Vaccine. No suitable data given.

USE - M1 is useful for manipulating glycoprotein production in an insect cell. M2 or M3 is useful for producing sialylated glycoprotein (claimed). The sialylated glycoprotein produced by the above mentioned methods are useful as pharmaceutical compositions, vaccines, diagnostics and therapeutics.

EXAMPLE - No suitable example given. (88 pages)

L7 ANSWER 4 OF 10 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2001:435294 HCAPLUS

DOCUMENT NUMBER: 135:41800

TITLE: **Recombinant** cells with altered intracellular sialylation pathways and their use in producing glycoproteins

INVENTOR(S): Betenbaugh, Michael J.; Lawrence, Shawn; Lee, Yuan C.; Coleman, Timothy A.; Palter, Karen; Jarvis, Don

PATENT ASSIGNEE(S): Human Genome Sciences, Inc, USA; Johns Hopkins University; Temple University; University of Wyoming

SOURCE: PCT Int. Appl., 182 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001042492	A1	20010614	WO 2000-US33136	20001207
W:	AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
RW:	GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG			

PRIORITY APPLN. INFO.: US 1999-169839P P 19991209

AB Methods for manipulating carbohydrate processing pathways in cells of interest are provided. Methods are directed at manipulating multiple pathways involved with the sialylation reaction by using **recombinant** DNA technol. and substrate feeding approaches to enable the production of sialylated glycoproteins in cells of interest. These carbohydrate engineering efforts encompass the implementation of new carbohydrate bioassays, the examination of a selection of insect cell lines and the use of bioinformatics to identify gene sequences for critical processing enzymes. The compns. comprise cells of interest producing sialylated glycoproteins. The methods and compns. are useful for heterologous **expression** of glycoproteins. Thus, the cDNA for a **human** sialic acid 9-phosphate synthetase which produces phosphorylated KDN and Neu5Ac from ManNAc-6-P and Man-6-P was **cloned** and sequenced. Sf9 cells infected with a baculovirus encoding this enzymes produced enhanced levels of sialic acids when the culture medium was supplemented with ManNAc.

REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 5 OF 10 MEDLINE on STN

DUPLICATE 1

ACCESSION NUMBER: 2001467579 MEDLINE

DOCUMENT NUMBER: PubMed ID: 11479279

TITLE: Molecular **cloning** of a unique **CMP-sialic acid synthetase** that effectively utilizes both deaminoneuraminic acid (KDN) and N-acetylneuraminic acid (Neu5Ac) as substrates.

AUTHOR: Nakata D; Munster A K; Gerardy-Schahn R; Aoki N; Matsuda T;

Kitajima K  
 CORPORATE SOURCE: Department of Applied Molecular Biosciences, Graduate  
 School of Bioagricultural Sciences, Nagoya University,  
 Nagoya 464-8601, Japan.  
 SOURCE: Glycobiology, (2001 Aug) 11 (8) 685-92.  
 Journal code: 9104124. ISSN: 0959-6658.  
 PUB. COUNTRY: England: United Kingdom  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 OTHER SOURCE: GENBANK-AB027414  
 ENTRY MONTH: 200110  
 ENTRY DATE: Entered STN: 20010830  
 Last Updated on STN: 20011015  
 Entered Medline: 20011011

AB 2-keto-3-deoxy-D-glycero-D-galacto-nononic acid (KDN) is a sialic acid  
 (Sia) that is ubiquitously **expressed** in vertebrates during  
 normal development and tumorigenesis. Its **expression** is thought  
 to be regulated by multiple biosynthetic steps catalyzed by several  
 enzymes, including **CMP**-Sia synthetase. Using crude enzyme  
 preparations, it was shown that mammalian **CMP**-Sia synthetases  
 had very low activity to synthesize **CMP**-KDN from KDN and CTP,  
 and the corresponding enzyme from rainbow trout testis had high activity  
 to synthesize both **CMP**-KDN and **CMP**-N-acetylneuraminic  
 acid (Neu5Ac) (Terada et al. [1993] J. Biol. Chemical, 268, 2640-2648). To  
 demonstrate if the unique substrate specificity found in the crude trout  
 enzyme is conveyed by a single enzyme, cDNA **cloning** of trout  
**CMP**-Sia synthetase was carried out by PCR-based strategy. The  
 trout enzyme was shown to consist of 432 amino acids with two potential  
 nuclear localization signals, and the cDNA sequence displayed 53.8%  
 identity to that of the murine enzyme. Based on the Vmax/Km values, the  
**recombinant** trout enzyme had high activity toward both KDN and  
 Neu5Ac (1.1 versus 0.68 min<sup>-1</sup>). In contrast, the **recombinant**  
 murine enzyme had 15 times lower activity toward KDN than Neu5Ac (0.23  
 versus 3.5 min<sup>-1</sup>). Northern blot analysis suggested that several sizes  
 of the mRNA are **expressed** in testis, ovary, and liver in a  
 tissue-specific manner. These results indicate that at least one  
**cloned** enzyme has the ability to utilize both KDN and Neu5Ac as  
 substrates efficiently and is useful for the production of **CMP**  
 -KDN.

L7 ANSWER 6 OF 10 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on  
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ACCESSION NUMBER: 2002:189008 BIOSIS  
 DOCUMENT NUMBER: PREV200200189008  
 TITLE: Sialylation of the Pasteurella multocida cell surface.  
 AUTHOR(S): Vimr, E. R. [Reprint author]; Lichtensteiger, C. A.  
 [Reprint author]  
 CORPORATE SOURCE: University of Illinois at Urbana-Champaign, Urbana, IL, USA  
 SOURCE: Abstracts of the General Meeting of the American Society  
 for Microbiology, (2001) Vol. 101, pp. 141. print.  
 Meeting Info.: 101st General Meeting of the American  
 Society for Microbiology. Orlando, FL, USA. May 20-24,  
 2001. American Society for Microbiology.  
 ISSN: 1060-2011.

DOCUMENT TYPE: Conference; (Meeting)  
 Conference; Abstract; (Meeting Abstract)  
 Conference; (Meeting Poster)

LANGUAGE: English  
 ENTRY DATE: Entered STN: 13 Mar 2002  
 Last Updated on STN: 13 Mar 2002

AB Bacterial pathogens belonging to the Haemophilus-Actinobacillus-  
 Pasteurella (HAP) group are obligate microparasites of the mammalian  
 oropharynx and can cause severe respiratory or invasive disease in

humans, domestic animals, and wildlife. Sialic acids are ubiquitous components of mammalian cell surfaces and serum glycoconjugates. At least one HAP member, Haemophilus influenzae, has been shown to mimic the host environment by phase-variation of its surface sialic acids. To our knowledge, no other HAP member has been shown to sialylate its cell surface. However, recent DNA sequencing of the Pasteurella multocida genome suggests this bacterium may encode functions for sialic acid catabolism, activation (synthesis of **CMP**-sialic acid), and glycosyl transfer ( $\alpha$ 2,6-sialyltransferase). To determine if P. multocida is capable of sialylation, the cell-free membrane fractions from two common serotypes (types A and D) were shown to sialylate endogenous acceptor(s) when provided with exogenous **CMP**-(14C)sialic acid. Confirmation that the transferred sialic acid was incorporated into the expected glycosidic linkage was obtained by demonstrating sensitivity of the label to digestion with **recombinant** Vibrio cholerae sialidase. The predicted absence of the biosynthetic genes for sialic acid synthesis suggests, as we have shown previously for H. influenzae, that P. multocida acquires free sialic acid from its host and then makes a metabolic decision between catabolism or activation for cell surface sialylation. That P. multocida may synthesize two **CMP-sialic acid synthetases**, one of which is encoded by the last gene of a sialic acid catabolic operon, suggests this HAP bacterium enjoys considerable flexibility in its sialometabolism, potentially accounting for its wide host range.

L7 ANSWER 7 OF 10 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2000:628244 HCAPLUS

DOCUMENT NUMBER: 133:218534

TITLE: **Human** glycosylation enzymes and cDNAs and their use in drug screening, diagnosis, and therapy  
Coleman, Timothy A.

INVENTOR(S): Coleman, Timothy A.

PATENT ASSIGNEE(S): Human Genome Sciences, Inc., USA

SOURCE: PCT Int. Appl., 115 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000052136	A2	20000908	WO 2000-US5325	20000301
WO 2000052136	A3	20001228		
W:	AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
RW:	GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG			
AU 2000033884	A5	20000921	AU 2000-33884	20000301
EP 1159406	A2	20011205	EP 2000-912096	20000301
R:	AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI			
US 6333182	B1	20011225	US 2000-516143	20000301
JP 2002537796	T2	20021112	JP 2000-602748	20000301
US 2002137175	A1	20020926	US 2001-984205	20011029
US 6783971	B2	20040831		
US 2004142442	A1	20040722	US 2004-759277	20040120
PRIORITY APPLN. INFO.:			US 1999-122409P	P 19990302
			US 2000-516143	A3 20000301
			WO 2000-US5325	W 20000301

AB The present invention relates to novel **human** glycosylation enzymes and isolated nucleic acids containing the coding regions of the genes encoding such enzymes. Also provided are vectors, host cells, antibodies, and **recombinant** methods for producing **human** glycosylation enzymes. The invention further relates to diagnostic and therapeutic methods useful for diagnosing and treating disorders related to these novel **human** glycosylation enzyme polypeptides. Thus, a **human** cDNA encoding a protein with significant sequence homol. to mouse **CMP** N-acetylneuraminic acid synthetase was **cloned** and sequenced. This gene was **expressed** primarily in colon tissue. Another **human** cDNA encoded a protein with significant sequence homol. to C. jejuni cytidine **sialic acid synthetase**. A third **human** cDNA encoding a protein with significant sequence homol. to E. coli N-acetylneuraminic acid aldolase was **cloned** and sequenced. This gene was **expressed** primarily in immune cells and tissues such as primary dendritic cells, monocytes, and bone marrow.

L7 ANSWER 8 OF 10 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2000:628243 HCAPLUS

DOCUMENT NUMBER: 133:233546

TITLE: Engineering of intracellular sialylation pathways for sialylated glycoprotein production

INVENTOR(S): Betenbaugh, Michael J.; Lawrence, Shawn; Lee, Yuan C.; Jarvis, Don; Coleman, Timothy A.

PATENT ASSIGNEE(S): Human Genome Sciences, Inc., USA; Johns Hopkins University; University of Wyoming

SOURCE: PCT Int. Appl., 145 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000052135	A2	20000908	WO 2000-US5313	20000301
WO 2000052135	A3	20040108		
W:	AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, RU, TJ, TM			
RW:	GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG			
AU 2000035083	A5	20000921	AU 2000-35083	20000301
JP 2003524395	T2	20030819	JP 2000-602747	20000301
EP 1399538	A2	20040324	EP 2000-913684	20000301
R:	AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI, CY			
US 2002142386	A1	20021003	US 2001-930440	20010816
PRIORITY APPLN. INFO.:			US 1999-122582P	P 19990302
			US 1999-169624P	P 19991208
			WO 2000-US5313	W 20000301
			US 2000-227579P	P 20000825

AB Methods for manipulating carbohydrate processing pathways in cells of interest are provided. Methods are directed at manipulating multiple pathways involved with the sialylation reaction by using **recombinant** DNA technol. and substrate feeding approaches to enable the production of sialylated glycoproteins in cells of interest. These carbohydrate engineering efforts encompass the implementation of new

carbohydrate bioassays, the examination of a selection of insect cell lines and the use of bioinformatics to identify gene sequences for critical processing enzymes. The compns. comprise cells of interest producing sialylated glycoproteins. The methods and compns. are useful for heterologous **expression** of glycoproteins. Thus, the cDNA for a **human** sialic acid 9-phosphate synthetase which produces phosphorylated KDN and Neu5Ac from ManNAc-6-P and Man-6-P was **cloned** and sequenced. Sf9 cells infected with a baculovirus encoding this enzymes produced enhanced levels of sialic acids when the culture medium was supplemented with ManNAc.

L7 ANSWER 9 OF 10 MEDLINE on STN DUPLICATE 2

ACCESSION NUMBER: 92112296 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 1309720  
TITLE: Identification of a genetic locus essential for capsule sialylation in type III group B streptococci.  
AUTHOR: Wessels M R; Haft R F; Heggen L M; Rubens C E  
CORPORATE SOURCE: Channing Laboratory, Department of Medicine, Brigham and Women's Hospital, Boston, Massachusetts.  
CONTRACT NUMBER: AI07061 (NIAID)  
AI22498 (NIAID)  
AI28040 (NIAID)  
SOURCE: Infection and immunity, (1992 Feb) 60 (2) 392-400.  
Journal code: 0246127. ISSN: 0019-9567.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199202  
ENTRY DATE: Entered STN: 19920308  
Last Updated on STN: 19990129  
Entered Medline: 19920218

AB The type III capsular polysaccharide of group B streptococci (GBS) consists of a linear backbone with short side chains ending in residues of N-acetylneuraminic acid, or sialic acid. The presence of sialic acid on the surface of the organism inhibits activation of the alternative pathway of complement and is thought to be an important element in the virulence function of the capsule. We showed previously that a mutant strain of GBS that **expressed** a sialic acid-deficient, or asialo, form of the type III polysaccharide was avirulent, supporting a virulence function for capsular sialic acid. We now report the derivation of an asialo capsule mutant from a highly encapsulated wild-type strain of type III GBS, strain COH1, by insertional mutagenesis with transposon Tn916 delta E. In contrast to the wild-type strain, the asialo mutant strain COH1-11 was sensitive to phagocytic killing by **human** leukocytes in vitro and was relatively avirulent in a neonatal rat model of GBS infection. The asialo mutant accumulated free intracellular sialic acid, suggesting a defect subsequent to sialic acid synthesis in the biosynthetic pathway leading to capsule sialylation. The specific biosynthetic defect in mutant strain COH1-11 was found to be in the activation of free sialic acid to **CMP-sialic acid**: **CMP-sialic acid synthetase** activity was present in the wild-type strain COH1 but was not detected in the asialo mutant strain COH1-11. One of the two transposon insertions in the asialo mutant COH1-11 mapped to the same chromosomal location as one of the two Tn916 insertions in the previously reported asialo mutant COH31-21, identifying this site as a genetic locus necessary for **expression** of **CMP-sialic acid synthetase** activity. These studies demonstrate that the enzymatic synthesis of **CMP-sialic acid** by GBS is an essential step in sialylation of the type III capsular polysaccharide.

L7 ANSWER 10 OF 10 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED.  
on STN

ACCESSION NUMBER: 92046726 EMBASE  
DOCUMENT NUMBER: 1992046726  
TITLE: Identification of a genetic locus essential for capsule  
sialylation in type III group B streptococci.  
AUTHOR: Wessels M.R.; Haft R.F.; Heggen L.M.; Rubens C.E.  
CORPORATE SOURCE: Infectious Diseases Division, Harvard Medical School, Beth  
Israel Hospital, Boston, MA 02115, United States  
SOURCE: Infection and Immunity, (1991) 60/2 (392-400).  
ISSN: 0019-9567 CODEN: INFIBR  
COUNTRY: United States  
DOCUMENT TYPE: Journal; Article  
FILE SEGMENT: 004 Microbiology  
LANGUAGE: English  
SUMMARY LANGUAGE: English

AB The type III capsular polysaccharide of group B streptococci (GBS) consists of a linear backbone with short side chains ending in residues of N- acetylneuraminic acid, or sialic acid. The presence of sialic acid on the surface of the organism inhibits activation of the alternative pathway of complement and is thought to be an important element in the virulence function of the capsule. We showed previously that a mutant strain of GBS that **expressed** a sialic acid-deficient, or asialo, form of the type III polysaccharide was avirulent, supporting a virulence function for capsular sialic acid. We now report the derivation of an asialo capsule mutant from a highly encapsulated wild-type strain of type III GBS, strain COH1, by insertional mutagenesis with transposon Tn916ΔE. In contrast to the wild- type strain, the asialo mutant strain COH1-11 was sensitive to phagocytic killing by **human** leukocytes in vitro and was relatively avirulent in a neonatal rat model of GBS infection. The asialo mutant accumulated free intracellular sialic acid, suggesting a defect subsequent to sialic acid synthesis in the biosynthetic pathway leading to capsule sialylation. The specific biosynthetic defect in mutant strain COH1-11 was found to be in the activation of free sialic acid to **CMP-sialic acid: CMP-sialic acid synthetase** activity was present in the wild-type strain COH1 but was not detected in the asialo mutant strain COH1-11. One of the two transposon insertions in the asialo mutant COH1-11 mapped to the same chromosomal location as one of the two Tn916 insertions in the previously reported asialo mutant COH31-21, identifying this site as a genetic locus necessary for **expression of CMP- sialic acid synthetase** activity. These studies demonstrate that the enzymatic synthesis of **CMP-sialic acid** by GBS is an essential step in sialylation of the type III capsular polysaccharide.

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L17 ANSWER 20 OF 22 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED.  
on STN

ACCESSION NUMBER: 92046726 EMBASE  
DOCUMENT NUMBER: 1992046726  
TITLE: Identification of a genetic locus essential for capsule  
sialylation in type III group B streptococci.  
AUTHOR: Wessels M.R.; Haft R.F.; Heggen L.M.; Rubens C.E.  
CORPORATE SOURCE: Infectious Diseases Division, Harvard Medical School, Beth  
Israel Hospital, Boston, MA 02115, United States  
SOURCE: Infection and Immunity, (1991) 60/2 (392-400).  
ISSN: 0019-9567 CODEN: INFIBR  
COUNTRY: United States  
DOCUMENT TYPE: Journal; Article  
FILE SEGMENT: 004 Microbiology  
LANGUAGE: English  
SUMMARY LANGUAGE: English

AB The type III capsular polysaccharide of group B streptococci (GBS) consists of a linear backbone with short side chains ending in residues of N- acetylneuraminic acid, or **sialic acid**. The presence of **sialic acid** on the surface of the organism inhibits activation of the alternative pathway of complement and is thought to be an important element in the virulence function of the capsule. We showed previously that a mutant strain of GBS that **expressed** a **sialic acid**-deficient, or asialo, form of the type III polysaccharide was avirulent, supporting a virulence function for capsular **sialic acid**. We now report the derivation of an asialo capsule mutant from a highly encapsulated wild-type strain of type III GBS, strain COH1, by insertional mutagenesis with transposon Tn916ΔE. In contrast to the wild- type strain, the asialo mutant strain COH1-11 was sensitive to phagocytic killing by **human** leukocytes in vitro and was relatively avirulent in a neonatal rat model of GBS infection. The asialo mutant accumulated free intracellular **sialic acid**, suggesting a defect subsequent to **sialic acid** synthesis in the biosynthetic pathway leading to capsule sialylation. The specific biosynthetic defect in mutant strain COH1-11 was found to be in the activation of free **sialic acid** to **CMP-sialic acid**: **CMP** -**sialic acid synthetase** activity was present in the wild-type strain COH1 but was not detected in the asialo mutant strain COH1-11. One of the two transposon insertions in the asialo mutant COH1-11 mapped to the same chromosomal location as one of the two Tn916 insertions in the previously reported asialo mutant COH31-21, identifying this site as a genetic locus necessary for **expression** of **CMP- sialic acid synthetase** activity. These studies demonstrate that the enzymatic synthesis of **CMP-sialic acid** by GBS is an essential step in sialylation of the type III capsular polysaccharide.

L17 ANSWER 21 OF 22 MEDLINE on STN  
ACCESSION NUMBER: 91152127 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 1825612  
TITLE: Glycolipids and glycosyltransferases in permanent cell lines established from **human** medulloblastomas.  
AUTHOR: Gottfries J; Percy A K; Mansson J E; Fredman P; Wikstrand C J; Friedman H S; Bigner D D; Svennerholm L  
CORPORATE SOURCE: Department of Psychiatry and Neurochemistry, University of Goteborg, St. Jorgen Hospital, Hisings Backa, Sweden.  
CONTRACT NUMBER: CA 32672 (NCI)  
NS 20023 (NINDS)  
R37 CA11898 (NCI)  
SOURCE: Biochimica et biophysica acta, (1991 Feb 5) 1081 (3) 253-61.  
Journal code: 0217513. ISSN: 0006-3002.

PUB. COUNTRY: Netherlands  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199104  
ENTRY DATE: Entered STN: 19910428  
Last Updated on STN: 19980206  
Entered Medline: 19910408

AB Medulloblastoma biopsies are heterogenous and might contain normal brain tissue, which limits the usefulness of such tumor material for biochemical analyses. We have, therefore, examined the gangliosides and their metabolism using the medulloblastoma cell lines. Daoy and D341 Med, cultured both in vitro and as xenografts in nude mice. The ganglioside patterns in the Daoy showed a switch from a high GM2, 70% (mol% of total ganglioside **sialic acid**) and low lactoseries gangliosides (2%) content in monolayer cultures, to a high proportion of lactoseries gangliosides (50%) and virtually no GM2 (1%) in xenografts, but an increased proportion of other a-series gangliosides. The D341 Med showed a similar change regarding the lacto-series gangliosides from 1% in suspension culture to 10% in xenografts. The activity of five glycosyltransferases, GM3, GD3, GM2, GM1 and LA2 synthases, did not parallel the ganglioside patterns and could not account for the noted variations therein. In the Daoy cell line the LA2 synthase as well as the GM2 synthase activity was relatively high in both culture systems, despite the marked difference in the **expression** of GM2 and the lactoseries gangliosides. These results suggest that environmental factors play a crucial role for the in vivo activity of the glycosyltransferases.

L17 ANSWER 22 OF 22 MEDLINE on STN  
ACCESSION NUMBER: 89043536 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 3055198  
TITLE: The K1 capsular polysaccharide of Escherichia coli.  
AUTHOR: Silver R P; Aaronson W; Vann W F  
CORPORATE SOURCE: Division of Bacterial Products, Food and Drug Administration, Bethesda, Maryland.  
SOURCE: Reviews of infectious diseases, (1988 Jul-Aug) 10 Suppl 2 S282-6. Ref: 18  
Journal code: 7905878. ISSN: 0162-0886.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
General Review; (REVIEW)  
(REVIEW, TUTORIAL)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 198812  
ENTRY DATE: Entered STN: 19900308  
Last Updated on STN: 19900308  
Entered Medline: 19881212

AB Epidemiologic, immunologic, and genetic evidence indicate that the K1 capsular polysaccharide confers invasiveness to Escherichia coli. The capsule, an alpha-2----8-linked homopolymer of **sialic acid** (NeuNAc), provides the bacterium with a physical antiphagocytic barrier. Structural similarities between K1 and **human** tissue components suggest that immune tolerance may also be a factor in pathogenesis of K1 disease. The molecular and genetic events involved in the synthesis and export of the K1 polysaccharide were examined. The **cloned** K1 genes encode at least 12 proteins involved in capsule biosynthesis. These genes appear to be coordinately regulated and functionally clustered. One cluster is associated with the synthesis and activation of NeuNAc and includes the gene encoding **CMP-NeuNAc synthetase**. This enzyme catalyzes the activation of NeuNAc to **CMP-NeuNAc**. A second region, encoding five proteins, is associated with translocation of polysaccharide to the

bacterial surface. The K1 polysaccharide is a poor immunogen in humans, and an understanding of the key reactions involved in K1 synthesis may help in providing an alternative to anticapsular immunity.

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(FILE 'HOME' ENTERED AT 11:03:05 ON 04 OCT 2004)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 11:03:35 ON 04 OCT 2004

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L1      185254 S SYNTHETASE?
L2      1 S "CMP SILAIC ACID"
L3      11 S "SILAIC ACID"
L4      0 S L1 AND L3
L5      69303 S "SIALIC ACID"
L6      603 S L1 AND L5
L7      21033 S "CMP"
L8      438 S L6 AND L7
L9      6727337 S CLON? OR EXPRESS? OR RECOMBINANT
L10     182 S L8 AND L9
        E COLEMAN T A/AU
L11     214 S E3
        E BETENBAUGH M J/AU
L12     412 S E3-E7
L13     613 S L11 OR L12
L14     7 S L10 AND L13
L15     4 DUP REM L14 (3 DUPLICATES REMOVED)
L16     29 S HUMAN AND L10
L17     22 DUP REM L16 (7 DUPLICATES REMOVED)
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